USER´S MANUAL

1.4 Version

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1. INTRODUCTION

In our commitment to the innovation and development of flow cytometry, Cytognos presents Infinicyt™, state-of-the-art software for data integration and multidimensional analysis of flow cytometry files.

This software opens a wide range of possibilities, as it can generate files with a great number of parameters from a single sample.

From our web site www.infinicyt.com, it is possible to download trial versions of the product or order the licensed version. Other materials are also available in this site, such us *Tutorials*, *Demo Files*, and a *Users Area* for *Upgrades* and on-line *Seminars*.

This *User's Manual* will explain in detail all the capabilities of this software and how to handle the great amount of information provided with the different tools.

Please contact us for any further information at supportinfinicyt@cytognos.com.

2. INSTALLATION AND SETUP

Installation instructions, including data about the minimum hardware and software requirements, can be found in this section. However, if possible, we recommend referring instead to the "Readme" file of the installation disc, which should contain similar information, but possibly more complete, up-to-date and/or adapted to the specific version of Infinicyt™ that has been delivered to you.

2.1. Licence Details

The standard licensed version of Infinicyt™ is delivered in a pack containing the installation disc, as well as one USB key per individual license. The program will work only if there is a USB key connected to one of your computer's USB ports. It is possible to install Infinicyt™ in several different computers, but it will work only in those to which a USB key is connected at that moment.

In case of malfunction of a USB key, we will replace it at no cost in exchange of the malfunctioning one. If the malfunction is the responsibility of the client, the key will be replaced at the cost of the key itself. In case of loss of the key, the client will have to purchase a new license, so we strongly recommend taking care of these keys.

If the use of USB keys represents an inconvenience for you, you can consult us about the possibility of using alternative licensing techniques, without hardware components.

Please feel free to contact supportinfinicyt@cytognos.com for any questions regarding network compatibility or any further aspects of the above points.

2.2. Minimum system requirements

- Operating System: Windows 2000, XP, VISTA and 7. 32 or 64 bits version.
- CPU: Intel Pentium, AMD, or equivalent. 32 or 64 bits version.
- RAM: 1GB (2GB recommended).
- At least 150 MB of hard drive free space to install the program.
- VGA compatible graphics card.

- Recommended graphics card compatible with OpenGl 1.3 or later, or with DirectX 9.0 or later, to visualize 3D diagrams.

- Preferably large screen over 20-21" with at least 1280 x 1024 pixel resolution. (24" recommended, or a double monitor).

Apart from the above system requirements, an extra limitation will be given by the characteristics of the data files: the more events and parameters you want to study, the higher computer requirements (RAM memory and processor) you will need.

These requirements should be considered approximate and an orientation. The exact behaviour of Infinicyt™ in a specific computer may depend on specific configuration factors which would be complex to document and on the type and size of the files to be treated.

For this reason, to determine for sure if Infinicyt™ will run in a specific computer with full user satisfaction, we recommend to install a demo version and test it with files as real as possible.

2.3. Installation procedures

Your installation CD-ROM is prepared to automatically start the installation as soon as it is inserted into your CD-drive. In that case, please follow the instructions of the program installer.

If it doesn't start automatically, the CD-ROM contains a file named *setup.exe,* please run it and follow the instructions to install the program. The user who is carrying out the installation process should have write permissions on the selected folder to install Infinicyt™.

At some point, the Infinicyt™ installation process will ask the user to install the USB key drivers, necessary for the correct operation of the program. The user should carry on this procedure and the Infinicyt™ installation will resume and conclude.

If these drivers are not installed, Infinicyt™ will not be able to recognize the USB key and the program will not work. The only case in which the user can safely cancel this secondary installation process is when he or she knows for sure that the drivers have already been installed, during a previous installation of Infinicyt™, or from any other software using the same licensing devices.

2.4. How to run Infinicyt™

When the installation process is concluded you can start to use Infinicyt™. First of all, you should connect the license key to any available USB port of your computer. After that, double click on the shortcut created on the Desktop, or on Start Menu->Programs->Cytognos->Infinicyt->Infinicyt.

You can also execute Infinicyt™ by double clicking on the file named "InfinicytBW.exe" placed in your Infinicyt™ installation folder (usually c:\Program Files\Cytognos\Infinicyt\).

2.5. Language and regional configurations

The current version of the software is available in English and Spanish. At the beginning of the installation, it is possible to choose the language used during the installation process, however the Infinicyt™ version which will be installed contains both languages. Infinicyt™ will be configured to run using the selected language during the installation process. The language can be later changed, in order to do that, double click to open the file named "Infinicyt.ini" placed in your Infinicyt™ installation folder (usually c:\Program Files\Cytognos\Infinicyt) and add the following line:

If you want to select English:

language=en

If you want to select Spanish:

language=es

If no language is selected, Infinicyt™ will use the language selected in the Windows language bar. If Spanish is selected in the language bar Infinicyt™ will run using Spanish, if any other language is selected then Infinicyt™ will run using English.

2.6. Uninstallation

The program can be uninstalled through the Windows normal uninstallation process (Control Panel / Add or Remove programs). Alternatively, the program can also be uninstalled by double-clicking on the file "Uninstall Infinicyt.exe" localized in the folder "Uninstall_Infinicyt" inside the Infinicyt™ installation folder.

In some cases, the uninstallation program may inform the user that it was not possible to remove some of the files that were installed along with the program. In that case the user should eliminate these files by hand.

It should also be noted that the Infinicyt™ uninstaller does not remove the drivers associated with the license USB key, because they may be used by other programs. The drivers can be manually uninstalled from Control Panel, Add or Remove Programs.

2.7. Support Services

The standard support service included in the price of the first year of license and renewable for an annual fee allows the user to refer to us his or her technical problems or questions by e-mail. The standard e-mail address to which technical user requests should be addressed is supportinfinicyt@cytognos.com The standard way of reply will also be e-mail, although Cytognos technicians may choose other communication methods if available and considered more appropriate for the case.

Users with special support needs, for whom the above coverage might be insufficient, can consult us about the possibility of improving this coverage, at our website www.infinicyt.com, by e-mail to supportinfinicyt@cytognos.com, or through their usual commercial representative.

Cytognos also offers a variety of training courses that can be tailored according to the customer's needs. Please contact us for more information about availability and pricing of these courses.

2.8. Upgrades

Users will receive newsletters with new possible updates in the address registered in the Users Area of the website www.infinicyt.com. Please, enter your data once you've received the license with your login user and password, for further communications.

From the Users Area, new upgrades can be downloaded as well as many other features and advantages are available.

2.9. Special considerations

Coulter-Cytomation users:
Infinicyt™ does not apply compensation to Log acquired values.
To see the compensation in Area and Lin parameters, you need to check log acquisition, to get the compensation matrix.

Sentinel Drivers:
If you are installing Infinicyt™ in a computer with DIVA software, which includes Sentinel drivers as well, please make sure that the version of Infinicyt™ Sentinel Drivers is higher; only in that case install the drivers.

3. MAIN SCREEN

When starting INFINICYT™, the first window to appear is the Main Menu Window with the basic functions of the software:

File Merge, to allow the user to merge flow cytometric data from different files (obtained from different immunostained tubes) into a single file, representing a complete immunostaining protocol of one sample.

Calculate Data (only included in advanced version). The user may calculate several characteristics of the cells which have not been directly measured, based on the knowledge of those characteristics in phenotypically identical cells which have already been measured.

File Editor, to modify certain parameters on FCS files, such as parameter's label or typing corrections.

File Export, to export data in other formats readable by external analysis software or database software.

Manual Analysis, with multiple options and possibilities for easy handling of files.

Advanced Analysis (not included in basic version), with different applications of automatic analysis that will be included in the following updates. In this version, Advanced Analysis includes an automatic recognition for BCR-ABL fusion protein kit.

In addition, the following extra options are available in the **Menu bar**:

File: With all the main functions of the software.

File/Exit: Exits from Infinicyt™ program. Alternatively, you can close the main window of the application or (Ctrl+Q).

Profile/Load: Before starting analyzing, the user may load a previously defined profile. A profile consists on a default configuration with different options regarding data statistics, visualization, graphs configuration and other advanced options.

Profile /Save: To save a profile configured or modified by the user.

Profile/Configure: To modify the values of the data statistics, visualization, graphs and other advanced options of the profile. See section 4.13.5.10

Help/Users Manual: Displays the User´s Manual.

Help/About Infinicyt™: Displays a single window with relevant information regarding the current version and installation of the program.

This user's guide assumes that the operator has a basic knowledge of flow cytometry techniques, as well as data interpretation for a correct use of this software.

4. MANUAL ANALYSIS

4.1. Introduction

The manual analysis mode allows the multidimensional study of single FCS files, files generated by new-generation cytometers, as well as n-dimensional files generated after the Merge+Calculate Data process.

The software includes a wide variety of analysis tools, plots and graphics, as well as printed reports, which allow user-friendly interpretation of obtained results.

The identification of different cell populations is carried out by drawing regions on the computer screen, which are assigned different names and colors, allowing classification as a hierarchic tree. The analysis panels are completely configurable by the user.

Results can be shown in Histograms, 2D-plots and multidimensional plots with different choices of transformation of the original parameters, logarithmic, compensated, non-compensated, or even SSC-expanded.

Statistics are exportable to different types of data bases and reports may be personally designed by the user, following the requirements of each study.

4.2. Getting Started on Manual Analysis

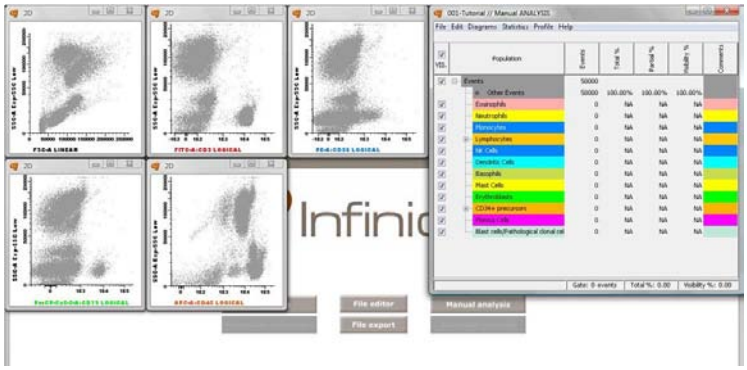
Once you have installed Infinicyt™ software on your computer you are ready to use it. Here is a short description of how to use the Manual Analysis, although all this actions are detailed later in different chapters:

A. OPEN INFINICYT

Launch Infinicyt™ by double-clicking on the icon that appears in the desktop, once it is already installed.

B. GO TO MANUAL ANALYSIS OPTION

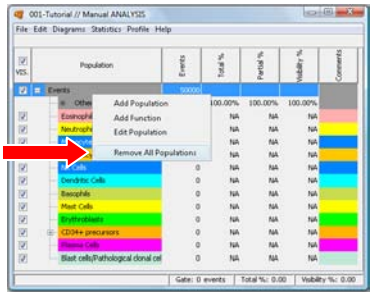
When you click on Manual Analysis, a file selection window will be opened in order to search for a sample file into the directory. Once you open a file, you get an example of a template with some diagrams and a Population Tree.



This Population Tree is an example and can be removed and built with different colours and labels.

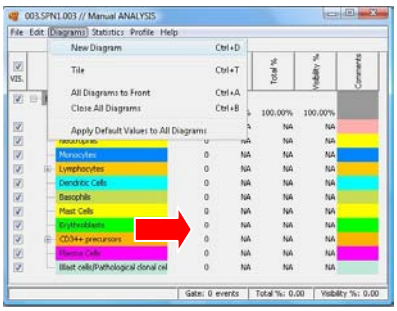
C. EDIT A POPULATION TREE

Right-click on any of the colored labels to obtain the population menu. In this menu you will find different options: Add Population, Add Function, Edit Population and Remove All Populations in order to customize your Population Tree.

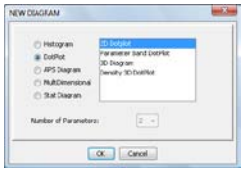


D. CREATE DIAGRAMS

To create a new diagram, go to the Diagrams/New Diagram or Ctrl+D. (On the Manual Analysis Window Menu Bar).

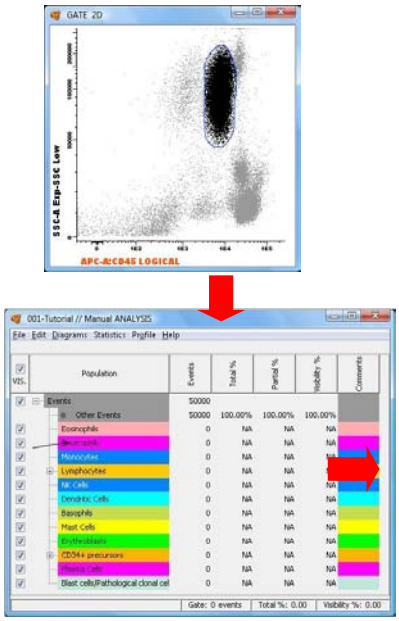


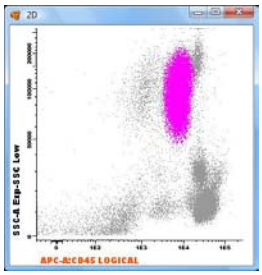
Select type of diagram



E. SAMPLE ANALYSIS

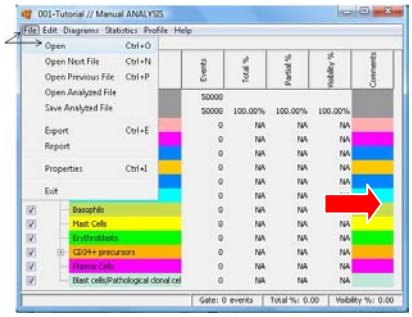
Draw a region with the mouse around the cells of interest. The region is automatically closed as soon as the user releases the mouse. The selected events will be shown in black in all plots. Go to the *Population Tree* and click on the label of the selected population. Then, the events will change the black color to the color selected.

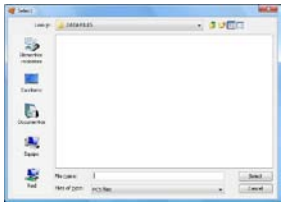




F. OPEN A NEW FILE

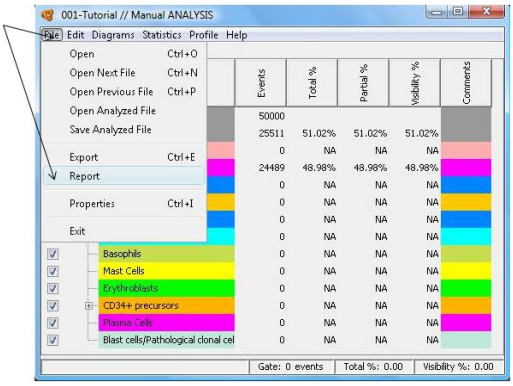
If you want to open another file just click on File/Open, then go to the directory and select another file.





G. REPORT

Go to File and click on Report. A page report opens with different components with standard information about the analyzed populations with the data included in the analyzed file.



The main components you can add to the report are the following:

1- Image, pictures or logos.

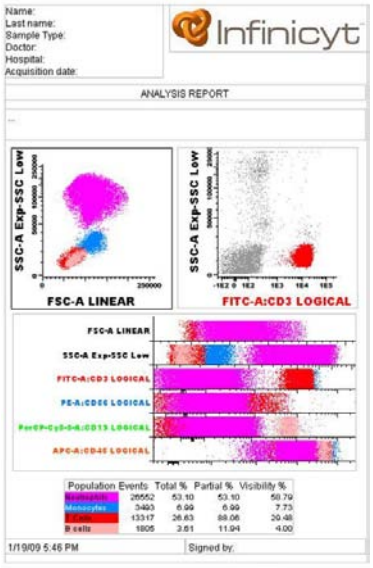
2- Text.

3- Data of the sample or from the patient.

4- Population tree with statistics.

5- Diagrams.

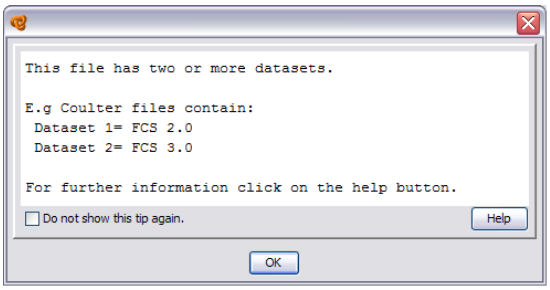
Right click on the report sheet and choose the component to use. Then you can right click on the component to configure it.



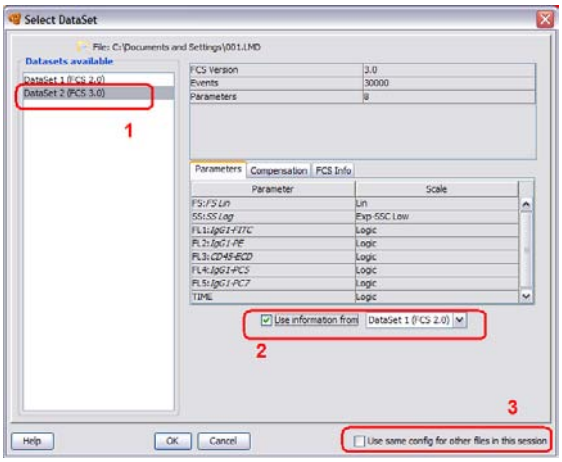
4.3. FCS files with more than one Dataset (Coulter Files)

Infinicyt™ supports all kind of FCS files that follows the *Data File Standards for flow cytometry* (www.isac-net.org). Some instruments, such us Coulter, generates FCS files with two different data sets: Dataset 1 (generated in version FCS 2.0) and Dataset 2 (generated in version FCS 3.0)

This new version of Infinicyt™ is able to open FCS files which includes two or more datasets. Once the file is selected, Infinicyt™ warns about this situation. The software asks the user which of the Datasets wants to open.


This file has two or more datasets.
E.g Coulter files contain:
Dataset 1= FCS 2.0
Dataset 2= FCS 3.0
For further information click on the help button.
Do not show this tip again.
Help
OK

Clicking on OK, the second dialogue box configures how to use these files during the whole session:



1.- The software as default uses the Dataset 2 (FCS 3.0), but Dataset 2 not always displays information of the labels and compensation.

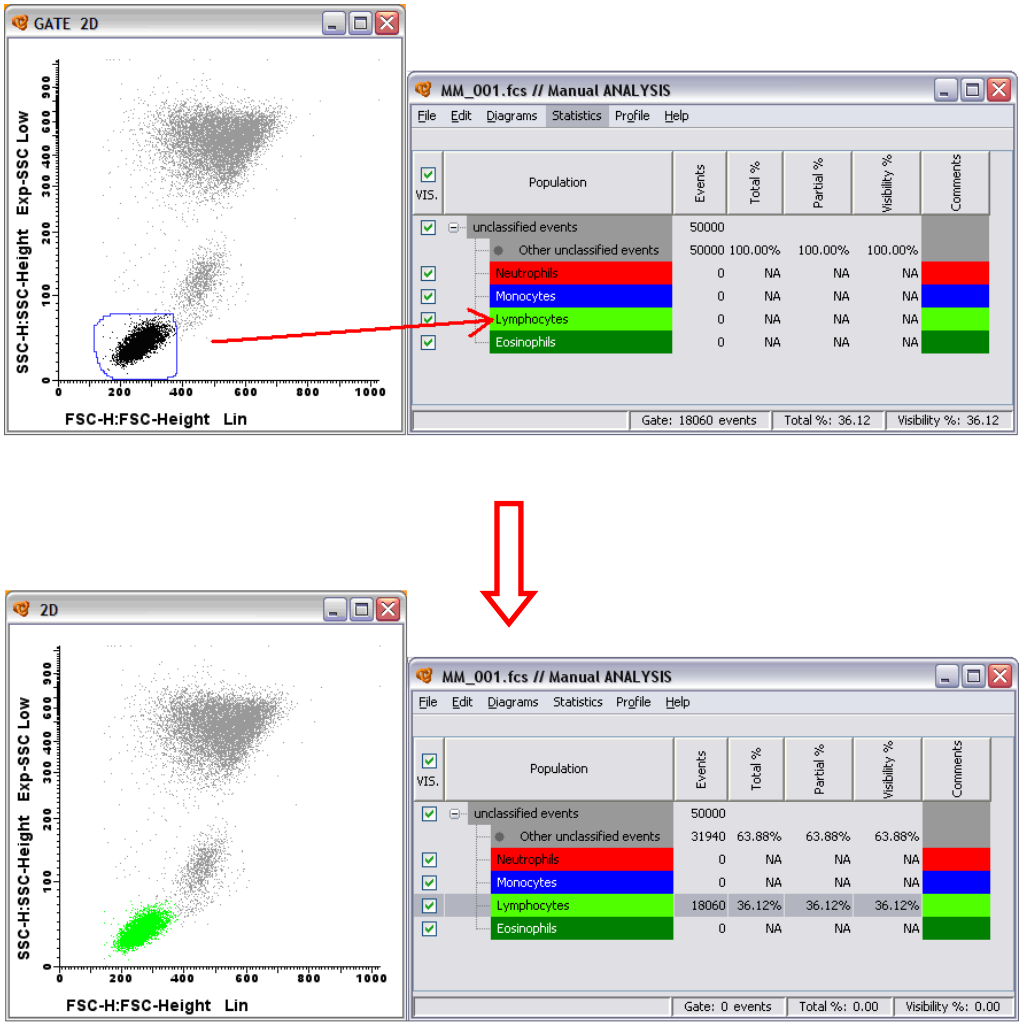
2.- Click on this tip to choose the Parameters labels and Compensation information from the Dataset 1.

3.- Use the same configuration for all the files you open in the same session to avoid repeating this message.

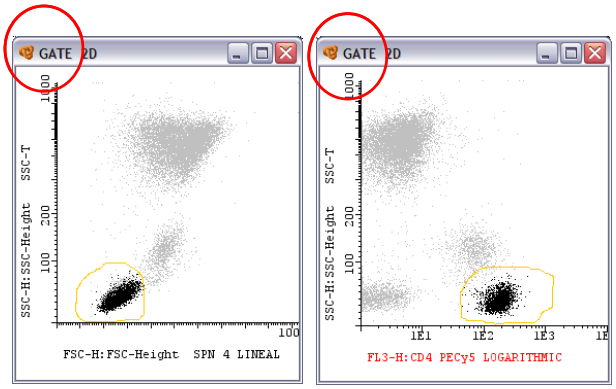
4.4. How to analyze and select populations

4.4.1. Assign label to populations

Once the user has opened a data file and the diagrams to perform the analysis, the selection of a population of interest is made by drawing a line with the mouse around the events cells. This line is automatically closed as soon as the user releases the mouse. Once the selection is closed, go to the *Population Tree* and click on the label of the population you are interested in. The events included in the region delimited by the line you drew will take the same color as the label.


GATE 2D
SSC-H:SSC-Height Exp-SSC Low
FSC-H:FSC-Height Lin
MM_001.fcs // Manual ANALYSIS
File Edit Diagrams Statistics Profile Help
VIS. Population Events Total % Partial % Visibility % Comments
unclassified events 50000
Other unclassified events 50000 100.00% 100.00% 100.00%
Neutrophils 0 NA NA NA
Monocytes 0 NA NA NA
Lymphocytes 0 NA NA NA
Eosinophils 0 NA NA NA
Gate: 18060 events
Total %: 36.12
Visibility %: 36.12
2D
SSC-H:SSC-Height Exp-SSC Low
FSC-H:FSC-Height Lin
MM_001.fcs // Manual ANALYSIS
File Edit Diagrams Statistics Profile Help
VIS. Population Events Total % Partial % Visibility % Comments
unclassified events 50000
Other unclassified events 31940 63.88% 63.88% 63.88%
Neutrophils 0 NA NA NA
Monocytes 0 NA NA NA
Lymphocytes 18060 36.12% 36.12% 36.12%
Eosinophils 0 NA NA NA
Gate: 0 events
Total %: 0.00
Visibility %: 0.00

To help the user during the analysis, if a gate has been drawn in any of the diagrams, before the label assignation this diagram will display the message GATE in the title as shown in the next figure.

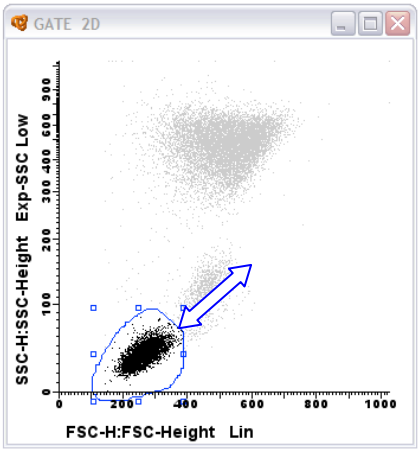


On Histograms, 2D DotPlots, Multigraphs or APS view (Automatic Population Separator), it is possible to draw regions with the mouse. The 3D Graphic is the only representation in which it is not possible to draw or select regions.

Each one of the operations you perform in the manual analysis can be Undone or Redone through these commands available in the Edit option of the *Analysis Window* menu (4.13.2.1).

4.4.2. Resize a region

Before clicking on the corresponding label to give a color to a region, it can be resized in the same DotPlot just by clicking inside the region. Then, you can drag, make it bigger or smaller or move the region with the mouse. Then, click again outside of the gate.



4.4.3. Redraw a region

Only one region can be drawn on each diagram. Therefore, if you draw a second region in a same plot, it will be considered a redraw and only the second one will apply.

Once a region is closed on a DotPlot, it can be redrawn just drawing a new one. The last one will replace the initial one.

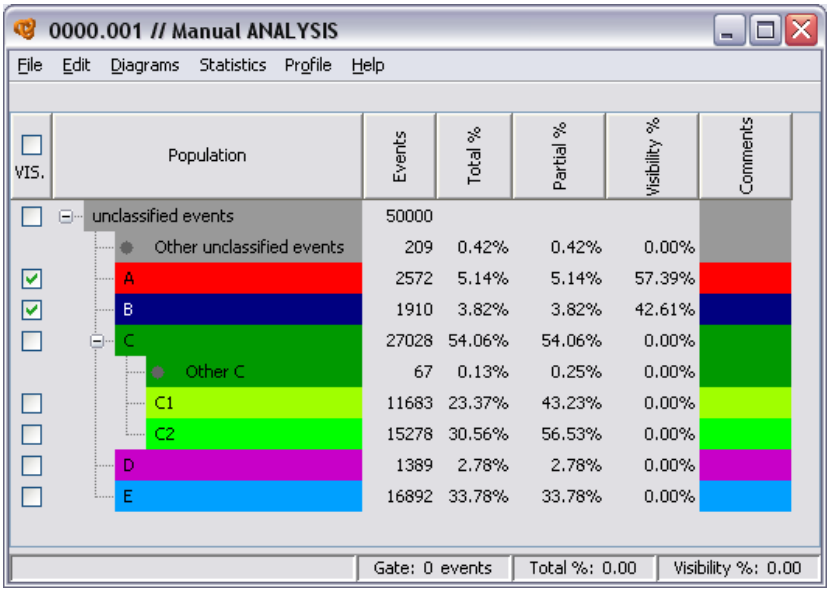
4.4.4. Consecutive gates (AND)

If the user considers that the made selection could be improved in a different graph, it is possible to draw consecutive regions in other DotPlots. In this case, only the events included in all the consecutive steps as the intersection, will be considered in the selection.

4.4.5. Population Tree description

As previously described, the program visualizes the results in two different ways: a table with populations and numerical results (Analysis Window) and a set of graphical representations configured by the user in independent windows. The former window shows a *Population Tree* with one line per population identified by the user, in general with color labels, where the color coincides with that of the dots or lines used to represent this population on the diagrams. For each population, the program provides the following information, as shown in the next figure:

A population is defined when we establish a group of events as an element in the hierarchy on the *Population Tree*. (Assign label to populations).

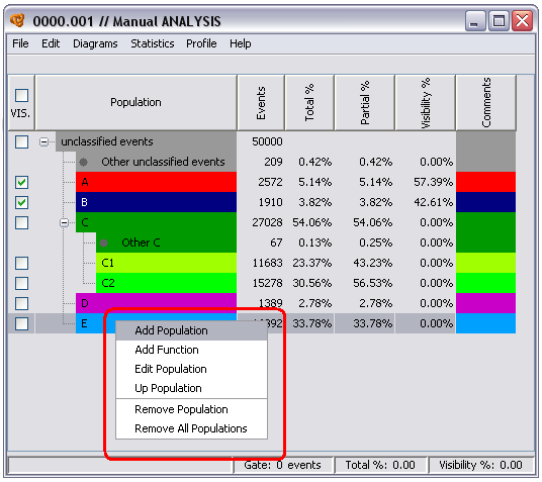


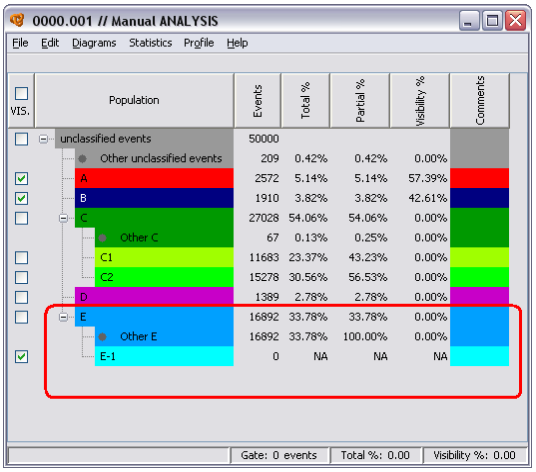
All the columns can be moved, or their order changed, by just clicking and dragging the heading of the column to the desired place.

The column **Population** contains the name or key that identifies the population whose data appears in the line. The color of this field coincides with that of the dots or lines representing this population in the graphics. The user can edit the populations chart.

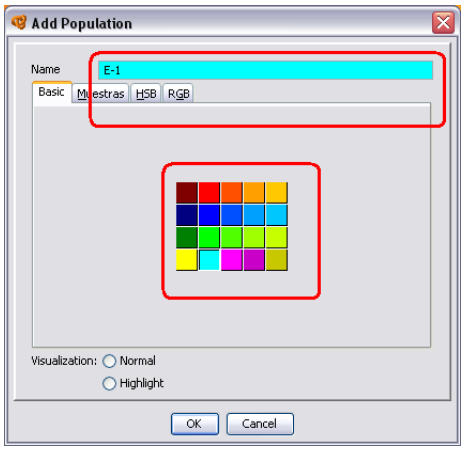
4.4.6. Create labels

To create a new population, right-click on the first label of "*Events*" and you will have the option to create a population, which will depend on the total cells. If you want your new populations to be a subset of a pre-existing one, then right-click on the pre-existing population, and create new one depending from the previously selected population.





Whether you **Add a population** or **Edit a population**, you will be able to write the name of the label you want in the box name and also choose its color by using the standard or advanced options for colors.



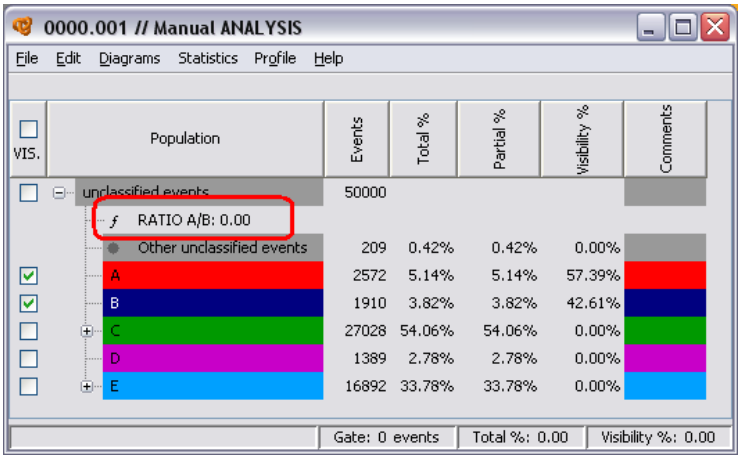
If you want to visualize your population with **Highlight**, you may enable this option in the *Edit Population* Dialogue Box. If you want to visualize populations with automatic highlight, whenever they represent less than a given percentage, then choose Profile/Configure/Graphs from the menu and define the maximum percentage as described in 4.13.5.10.

4.4.7. Drag and drop labels

It is possible to move a label to be included as a subpopulation of a pre-existing one, just clicking and moving the label without releasing the mouse, as a drag and drop tool. With **Up Population** and **Down Population** in the right click of the label you may change the order of the populations of the tree, and the population will move one place up or down depending on the command you select.

4.4.8. How to add a Function in the Population Tree

With **Add Function** you will go directly to a window similar as the one shown on chapter 4.13.5.10 (Profile/Configure/Statistics) where you can create your own function to relate populations. The new function added will not be a new column with values for each population, but it will be a unique value shown as a new population.



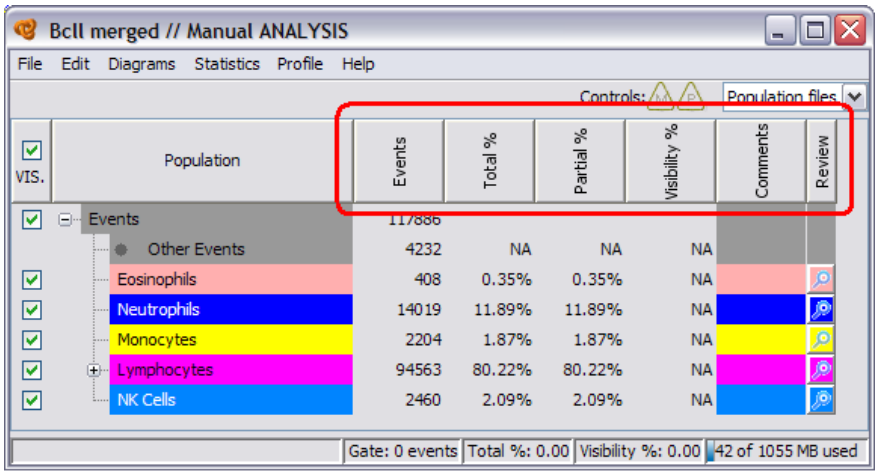
4.4.9. How to remove population labels

You can delete a population from the Population Tree by selecting the option **Remove population** in the right click menu of the label, or even delete all of them from the tree with **Remove All Populations.**

4.4.10. How to select, show or hide a population (VIS Column)

The column VIS., located in the left part of the window, contains checkboxes indicating whether the corresponding population must, or must not, be visible in the graphical panels. If the box is dashed, the population appears, otherwise it is not shown.

4.5. Statistic Information



4.5.1. "Events" column

This column indicates the total number of events counted by the program for the population identified in the "GROUP" column.

4.5.2. "Total %" column

This column will indicate the percentage that the events of this population represent with respect to the total number of events included in the data file (or file chosen in the pop-up list if we are analyzing a merged file).

The decimal separator and the number of decimal places can be changed at Profile/Configure/Statistics, and it will apply to all the values shown in all the columns of the control Analysis Window and Report.

4.5.3. “Partial %” column

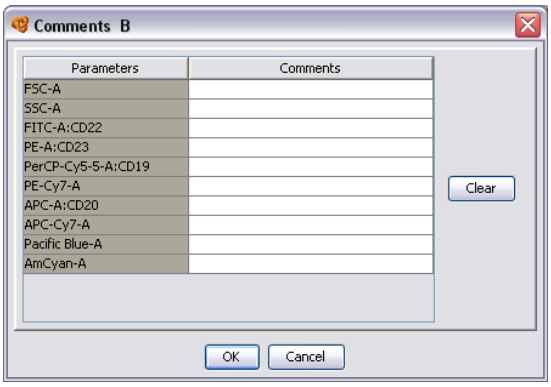
This column will indicate the percentage of events attributed to the considered population, relative to the total number of significant events of the population that can be considered the “hierarchical superior” of the population under consideration.

4.5.4. “Visibility %” column

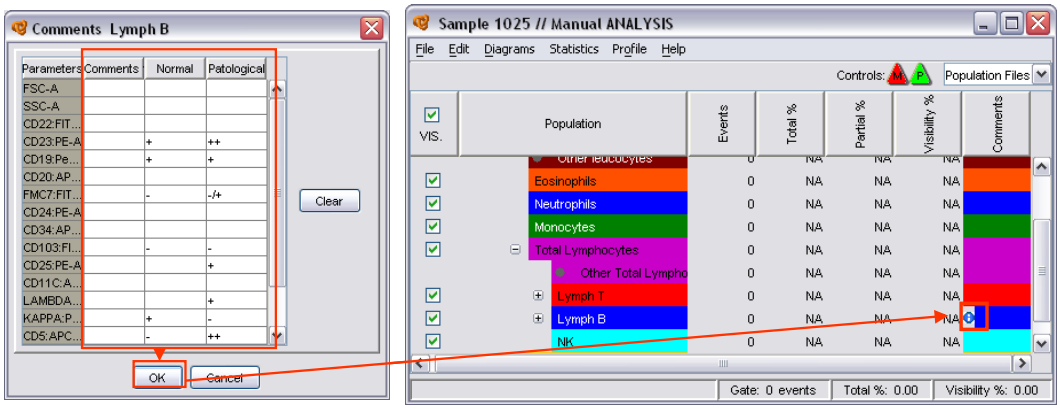
This column will indicate the relative percentage of the populations visualized at that moment in the analysis, the value 100% representing the sum of all shown populations.

4.5.5. “Comments” column

By clicking on each of these labels from this column the user may write a comment about the expression of a parameter in a population.



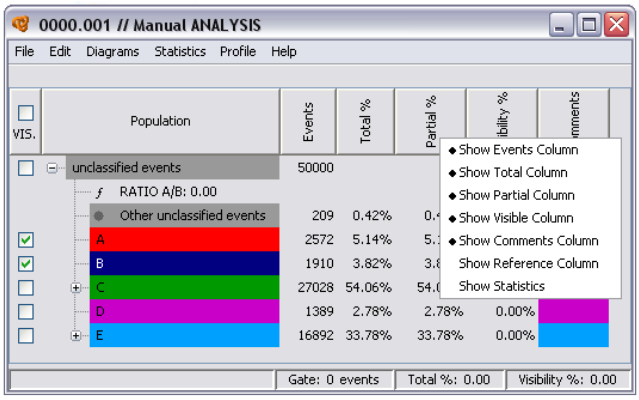
This comment will be saved and will appear any time you click on each colored label, and it will be saved to the printed report.



The number of comment columns can be chosen as explained on chapter 4.13.5.10, in the section of Analysis.

4.5.6. Statistics columns

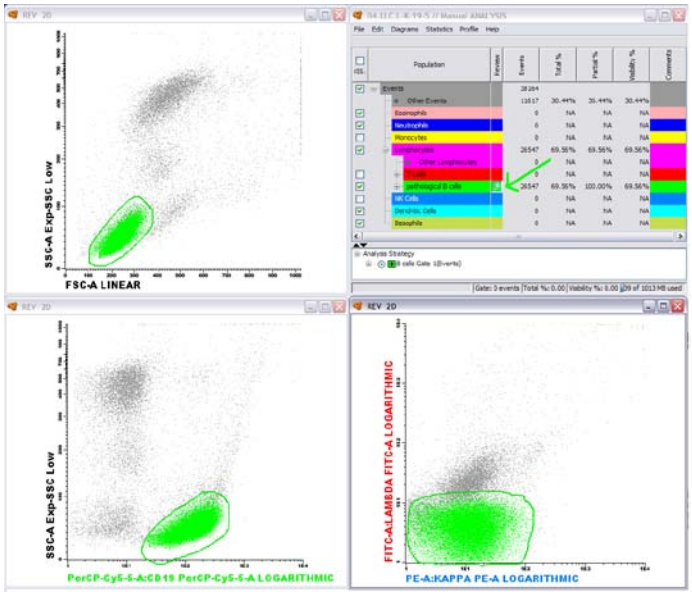
As a default all the columns previously described, except these statistics columns, will be visible. Nevertheless, at any time, clicking with the right button of the mouse, this information can be hidden or shown.



When the user activates “show statistics”, there will appear as many columns in the dialogue box as the number of parameters measured in the data file. The columns can be closed (◄) or opened (►) to visualize the data about Mean, SD and CV, or to visualize the comments if they exist.

4.5.7. Review Column (Rev)

On Infinicyt v1.4 you can review your gating strategy. Any time an analysis is performed, it will appear a lens that you can click to review the regions applied to get that population. See chapter 4.10.1for more info.



4.6. Types of graphic representations

The representation and manual analysis of cytometric files is performed by several graphical representation windows, which can be, in the current version, of the following types described in this chapter.

Each one of these diagrams is included in its own individual window, which can be moved throughout the screen, or amplified, reduced or hidden according to the user's needs. Since they can be amplified to full-screen size, the diagrams can provide a very detailed view of the different event sets. When the program is closed, it remembers the characteristics of all the diagrams, and when it is restarted it regenerates automatically the diagrams with the same characteristics that they had at the closing of the last session. To eliminate a diagram, just close its window.

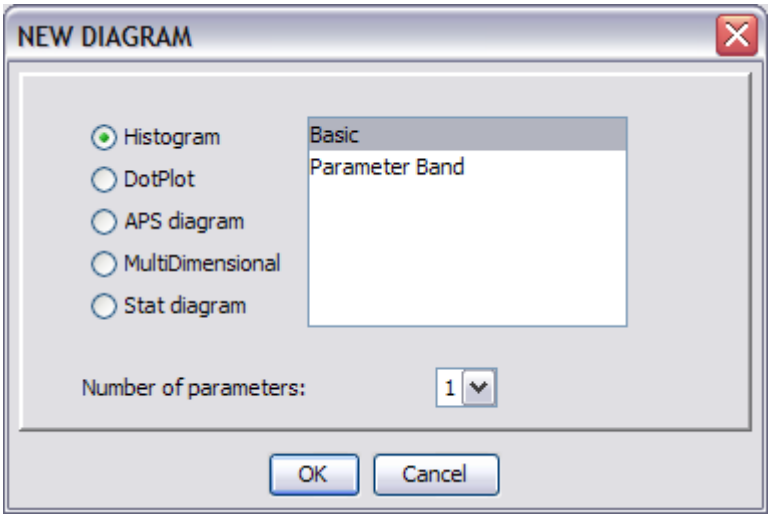
All diagrams contain an internal configuration menu, with a right click in the middle of the graph. The complete menu in the right click is explained in detail in chapter 4.7.

To create a new graphic

Click **Diagram** in the menu of the *Analysis Window Menu Bar* and choose **New diagram** or Ctrl+ D (see 4.13.3.1 **Diagrams menu**). Then, the user may choose between Histograms, DotPlots, APS views, MultiDimensional graphs or Statistical graphs, and choose the number of dimensions to be represented.

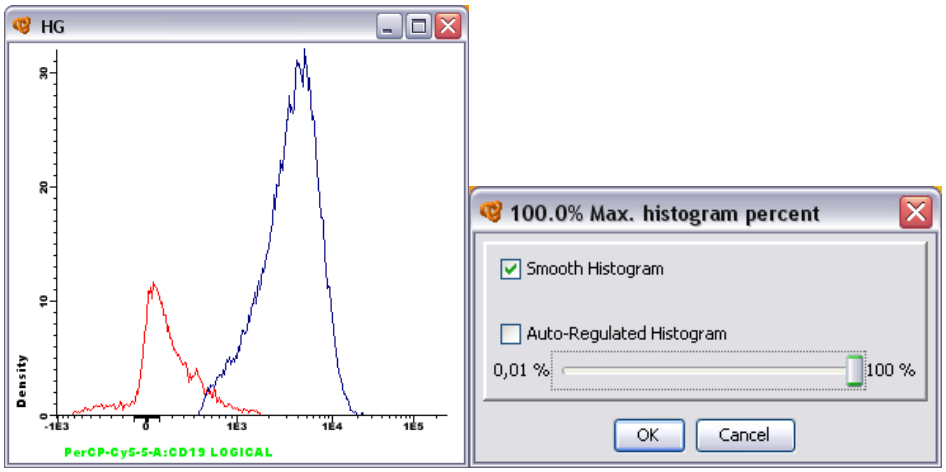
4.6.1. Histogram

Graphical display of tabulated frequencies. We can choose:



-Histogram

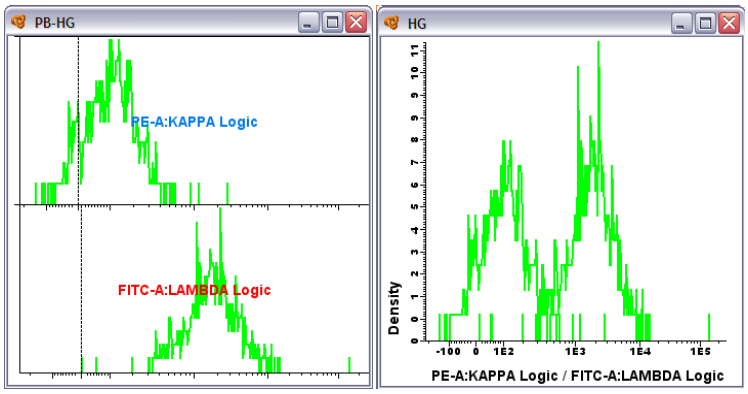
Graphic representation of one or more parameters in the horizontal axis. The number of events is represented in the auto-scaled vertical axis. There is a possibility of a manual configuration of the scale, by **right-clicking** on the Histogram and choosing **Diagram Configuration/Histogram Configuration**.



Smooth Histogram is a tool to improve the visualization of the peaks.

Auto-regulated Histogram: you can auto-regulate the maximum value to show on the Y axes. This is a useful option when the high values on the Y axes don't let to clearly visualize the lower values of the other peaks.

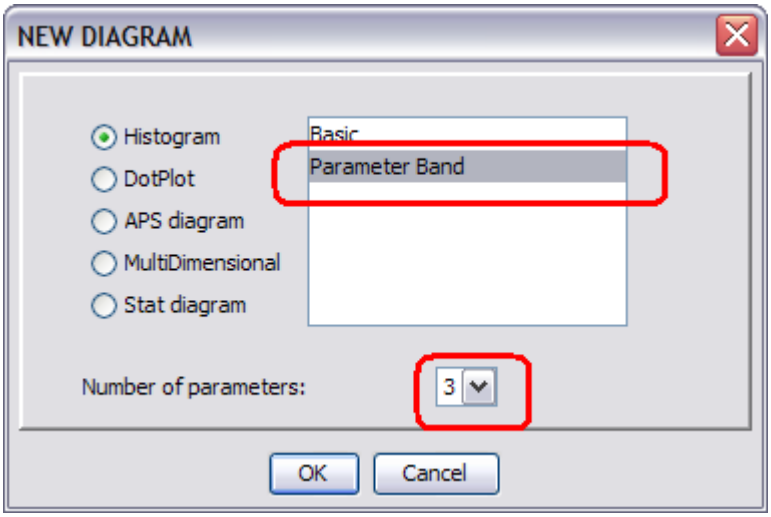
We can choose the number of dimensions to be represented in the same histogram. For example, we can overlap two parameters of the same population in two different ways as shown in the next figure.



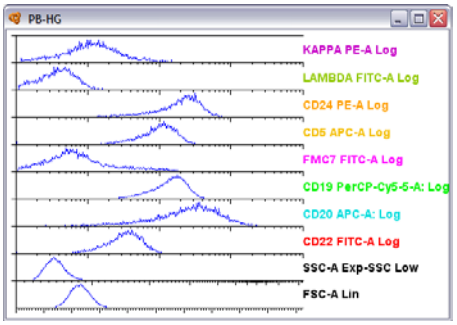
The first graphic is a **Parameter Band Histogram** of two dimensions.

The second one is a **Histogram** of two dimensions. The scale in this case will be shown only if both parameters include the same scale. If they are not the same, no scale will be shown.

Both diagrams can be obtained from **New Diagram/Histogram** on the Manual Analysis Window Menu Bar and then, the user selects as many parameters as desired to be represented in the same graphic.



The user can choose the number of dimensions with the only limitation of the number of parameters measured.

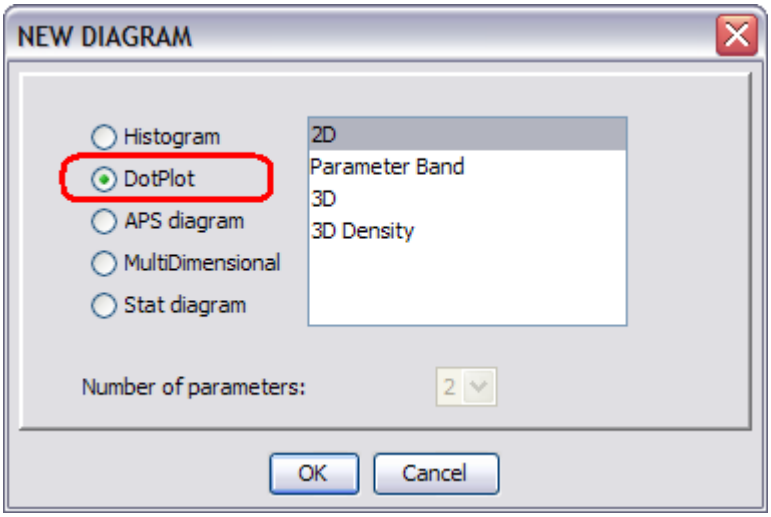


To increase the number of bands, decrease, or change the order once it is done, it is possible to do it by right clicking in the diagram and the choose Diagram Configuration /Axes configuration.

4.6.2. 2D DotPlot

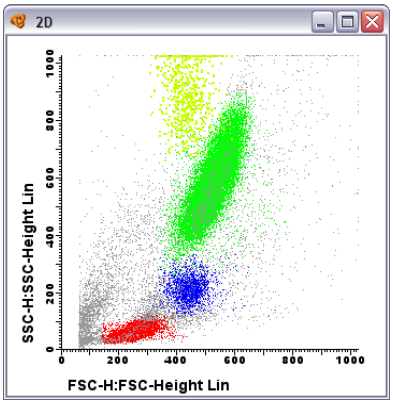
A **DotPlot** is a statistical chart consisting of a group of data dots plotted on a simple scale. DotPlots are one of the simplest plots available. They are useful for highlighting clusters and gaps, as well as outliers.

We can choose different types of DotPlots:



-2D Dot plot

It is a Graphical representation of events (cells or particles) in two dimensions. The value on each of the two dimensions (parameters) defines the location of the event in the plot.

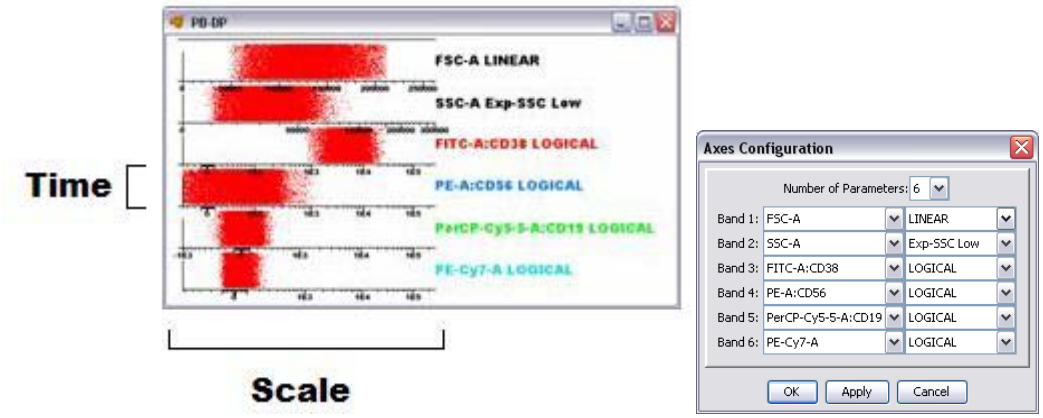


To change the parameters or the scale, click on the label in the axis and you will have available all the possible parameters to show, or the different scales.

As exposed in chapter 4.7 each diagram contains an internal menu which can be obtained right-clicking in the middle of the diagram.

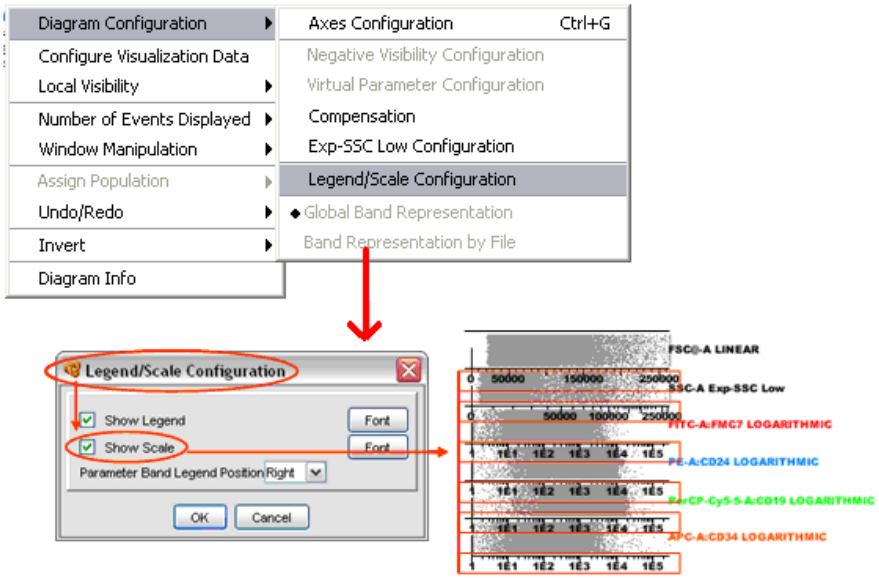
-Parameter Band DotPlot:

As described in the Parameter Band Histogram, the user can select as many different bands as parameters desired to represent the events in a dot plot. **Each band will represent the expression of the parameter X in those cells with the Time parameter of each band in the Y axes.** The events will appear in the Time scale of each parameter in the order they were acquired.



These parameter band representations are especially useful to visualize the whole phenotype of a population in a merged data file containing all the parameters in the same file.

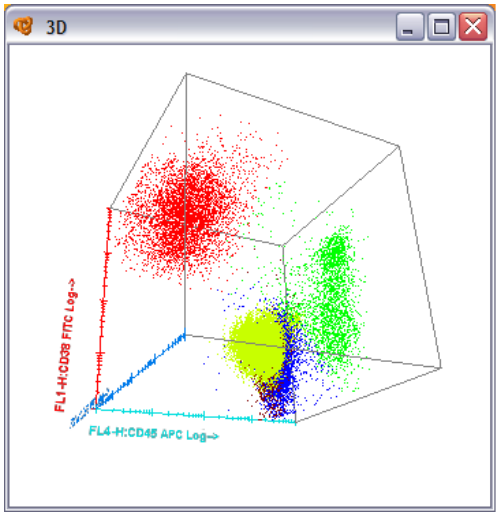
The scale of each band can be represented in the X axes. If the user prefers to hide the scale, right click on the diagram, choose Diagram Configuration/ Legend and Scale Configuration and choose or not “Show Scale”.



The complete menu in the right click is explained in detail in chapter 4.7.

-3D Graph:

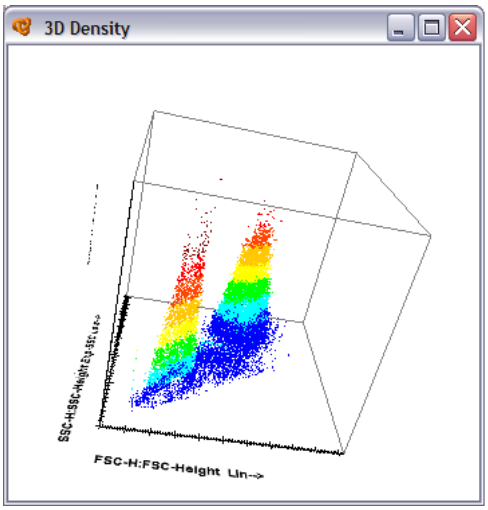
Graphical representation of events (cells or particles) in three dimensions. The value on each of the three dimensions (parameters) defines the location of the event in the diagram.



3D Diagrams can be resized clicking and moving the scroll of the mouse. It can also be rotated in all directions, and also with the numeric keyboard can be rotated with the numbers 2, 4, 6 or 8.

-Density 3D DotPlot

This diagram represents a 2D DotPlot with a third axe where the density of events in the 2D plot is represented.



4.6.3. Automatic Population Separator Diagram

This graphical representation is an automatic separation of the events, where the software is able to distinguish as many cell clusters as possible, analyzing all the different choices of parameter combinations, based on *Principal Component Analysis*.

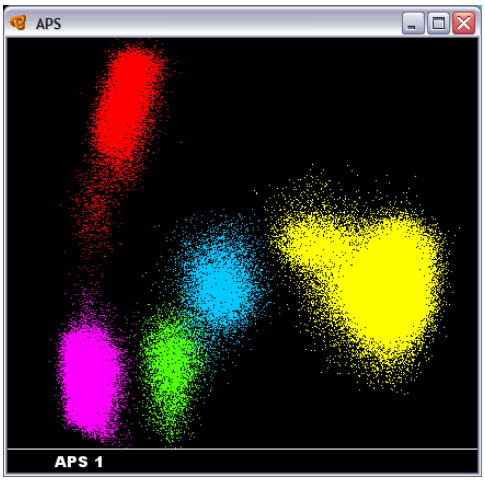
The parameters represented in these APS graphics are not a real measured parameter. They are defined by calculations of different percentages of each parameter for their contribution to the most optimized separation of clusters.

In the dialogue box of Diagram/New Diagram from the Manual Analysis Window Menu Bar, you will find the APS Graph.

-APS graph

The aim of this representation is allow the user to be able to distinguish in these graphics as many different populations as possible (especially useful when working with a high number of parameters) for a further characterization of these populations in the conventional 2D graphics. Usually 2D graphs can not give a good separation of clusters and a big number or diagrams with different combinations is needed to get the separation of all the groups.

APS shows different views. By default Infinicyt™ shows APS-1 view, which is the best combination for population separation. The APS-1 view shows the first versus the second Principal Component. In order to change to a different combination of parameters (to obtain the second one or third view), right-click on the axes and choose a different one. The number of possible APS views is the same as the number of parameters in the file.

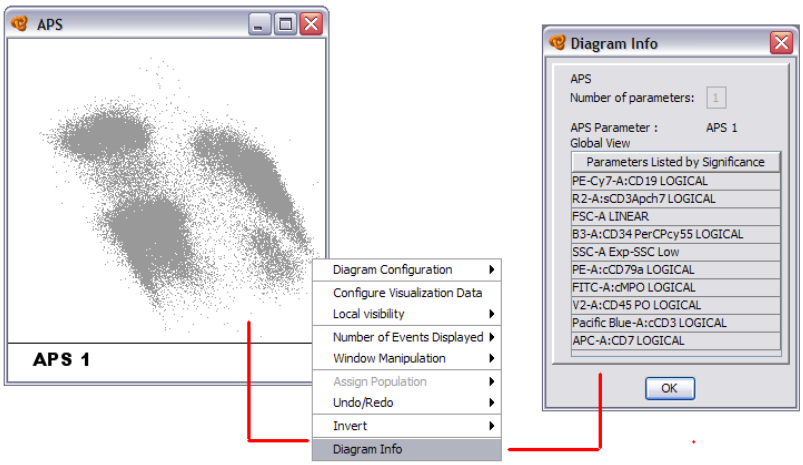


WARNING: if you are working with a merged file, in which you have null values (parameters not measured in all the tubes), in some tubes for some parameters, these events won't be shown. The correct way of showing the APS view in a merged file is configuring it only with common markers. The software will automatically do it, but it is

possible to change the configuration of the parameters (right click, Diagrams Configuration/ APS parameter Configuration).

APS Diagram Info

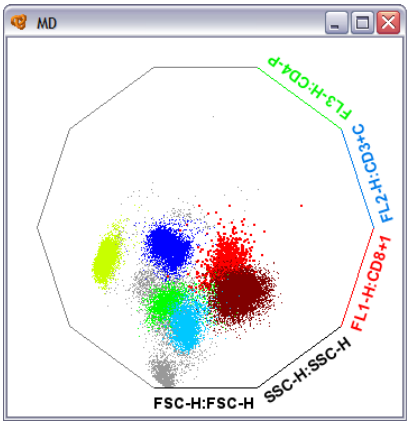
Interesting information of the APS is to know how is the contribution of each of the parameters to the separation of the clusters. You can get this information right-clicking in he graph and choosing Diagram Info. You will obtain the list of parameters in the order of contribution. It means that the first parameter in the list is the most important one to get a good separation from one group to the others.



4.6.4. MultiDimensional Graph

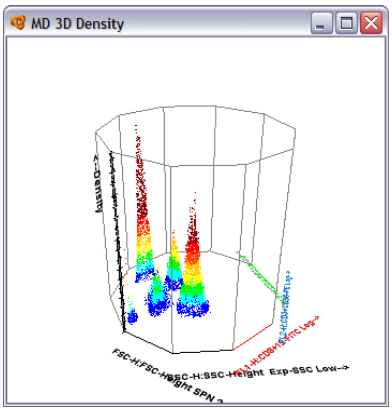
-Multidimensional Graph

Graphical representation of the events (cells or particles) in *n* dimensions. The user can choose the number of dimensions with the only limitation of the number of parameters measured. The value on each of the dimensions (parameters) defines the location of the event in the diagram. The main advantage of this representation is that the user may get a better separation of populations than with any other diagram, except APS which will do the separation automatically.



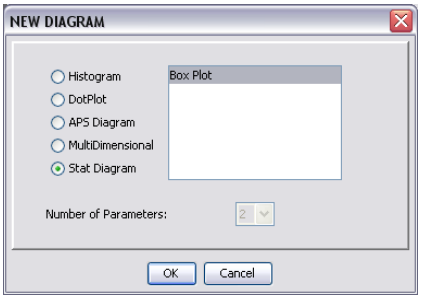
-Density 3D Multidimensional Graph

In the same way of the 2D Dot Plot there is an additional axes to represent density of the populations.

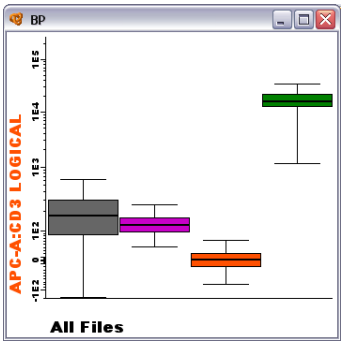


4.6.5. Statistical Diagrams: BoxPlot

A new statistical representation available is the Box Plots. If you click on **Diagram/New Diagram** on the Manual Analysis Menu Bar, you can choose the box plot in the **Stat Diagrams** option.



Then, you will obtain a diagram when you represent in the X axe all the populations visible in that moment in the population tree and in the Y axes you can choose the parameter to represent.



The box itself contains the middle 50% of the data. The upper edge (hinge) of the box indicates the 75th percentile of the data set, and the lower hinge indicates the 25th percentile. The range of the middle two quartiles is known as the inter-quartile range.

The line in the box indicates the median value of the data.

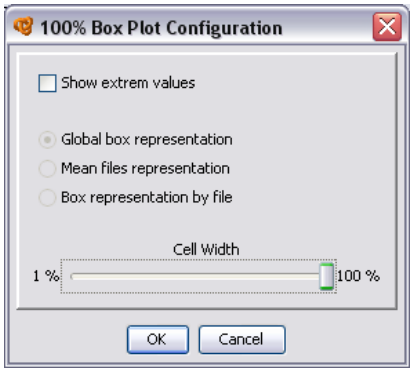
If the median line within the box is not equidistant from the hinges, then the data is skewed.

The ends of the vertical lines or "whiskers" indicate the minimum and maximum data values, unless outliers are present in which case the whiskers extend to a maximum of 1.5 times the inter-quartile range.

The points outside the ends of the whiskers are outliers or suspected outliers.

With a right click on the diagram, you can go to Diagram Configuration / Box Plot configuration and find several options to display the graph, specially useful when you are working with merged files.

If you choose to show extreme values, you will visualize the outliers.



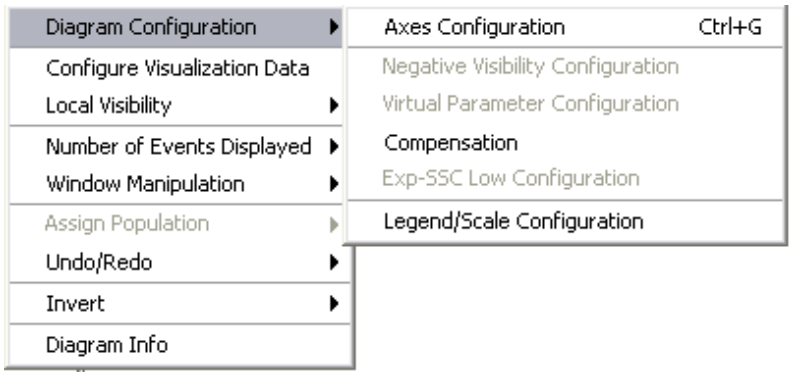
4.7. Diagrams right click menu

By right-clicking in the middle of the diagrams, we will obtain the following menu:

All these options will allow us to configure LOCALLY the display of the graphs.

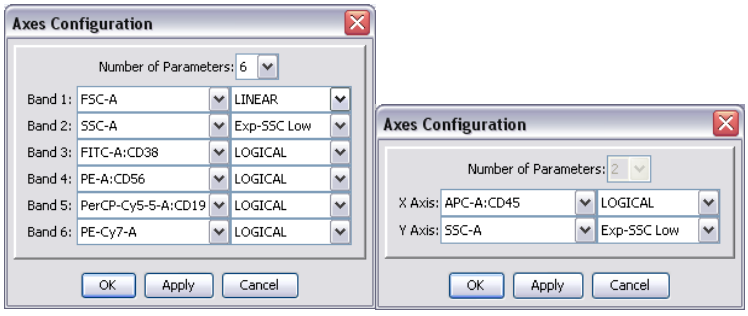
To apply a change globally to all the graphs, then go to the Manual Analysis Window Menu Bar and choose configure, Diagrams.

4.7.1. Diagram Configuration



4.7.1.1. Axes Configuration

Through this command the user may configure the parameters on each of the axes of the graphic (see figure).

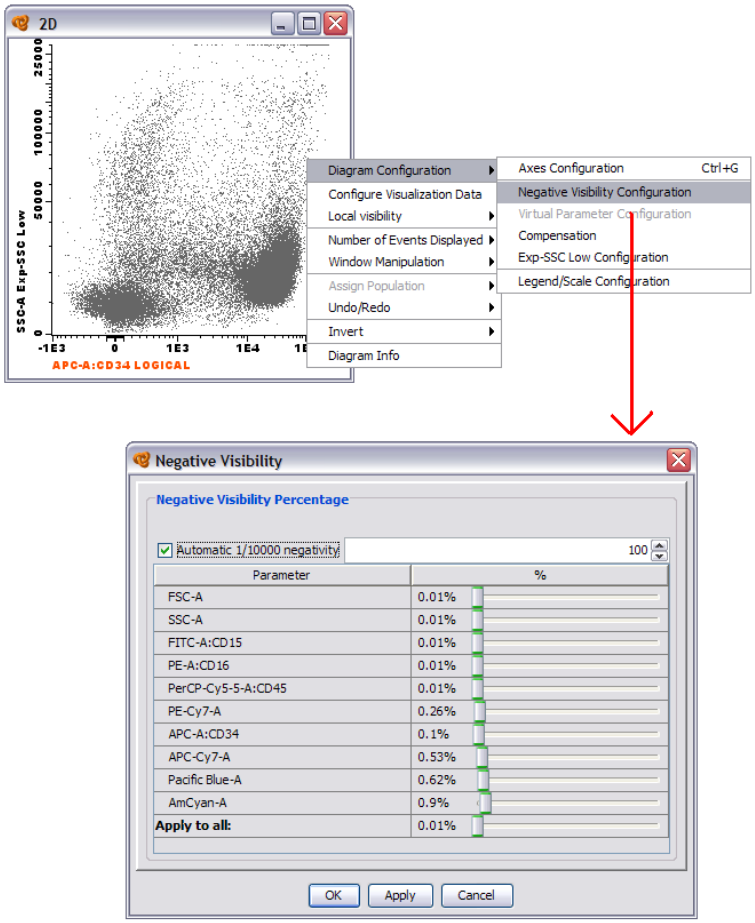


4.7.1.2. Negative Visibility Configuration

Whenever the FCS file has been obtained from an instrument with electronic compensation, negative values that are usually close to the axes may be generated.

This option allows visualizing the negative part of the scale to study the events shown on that region.

When you click this option, the window with the negative visibility percentage is displayed, and you can modify the values. This is the same window that appears in profile configuration, and this information will be saved as part of the profile.



4.7.1.3. Virtual Parameter Configuration

Though this option you obtain the Virtual Parameter Configuration window in the profile. See chapter 4.13.5.10 in the section of *Virtual parameters*.

In this window you can create new parameter as a combination of others and you can choose which calculation you want to represent (e.g. dividing a parameter by a constant number, rest one parameter from another…)

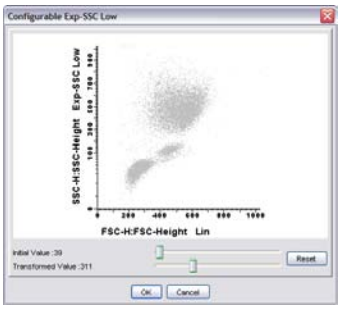
4.7.1.4. Compensation

This option shows the information of the compensation values which is stored in the data file. These values can be changed manually and be saved as…but you can always restore the original values.

It is also possible to choose a stored compensation and change the values to modify it.

4.7.1.5. EXP-SC Low Configuration

This option gives the possibility to expand the SSC-parameter with the values desired by the user. When clicking on it, the SSC plot will appear with two scrolls in the lower part of the graph to regulate the part of the scale you want to amplify.

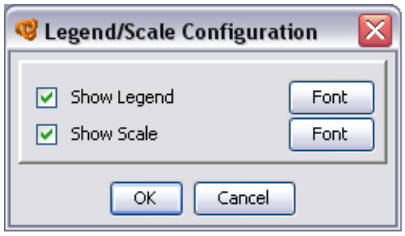


Default EXP-SSC Low: If you click this option in the DotPlot menu, a default mathematical transformation will be applied to the SSC parameter of the file to improve the separation between populations in the lower part of the SSC scale.

4.7.1.6. Font Legend/Scale configuration

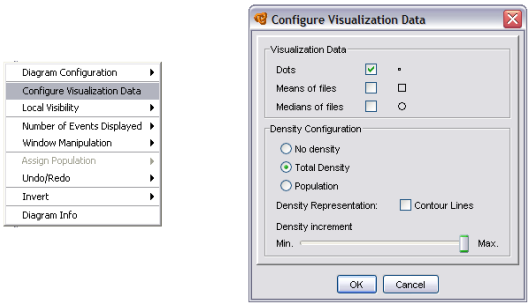
This option allows controlling the appearance of the parameter label. The user may change the appearance of the parameter labels by changing the font, size, bold, Italics…

If you want to have no legend in the parameter label, disable **Show Legend or Scale** in this window.

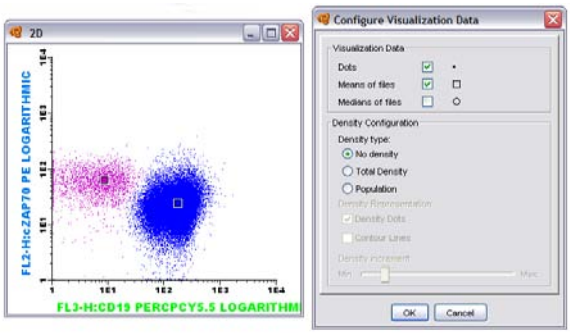


Font option allows controlling the size and appearance of numbers shown in the scale. If you want to show no scales and legend, just unclick both.

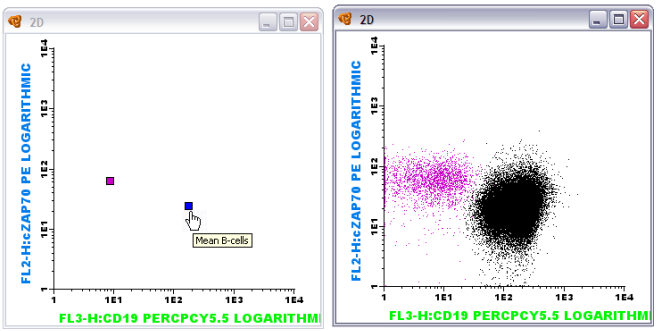
4.7.2. Configure Visualization Data



Visualization data: In the dialogue box the user can select **dots**, **mean** value or **median** value or a combination of some of them (dots and Mean), as you can see in the following picture.



If you draw a gate around a mean or median dot, all the events contributing to this value will be selected. Moreover, as shown in the following picture, clicking on the median or mean dot, the entire population is selected as well:

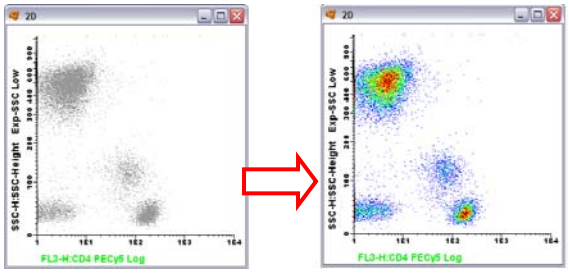


-Density Configuration: Density type

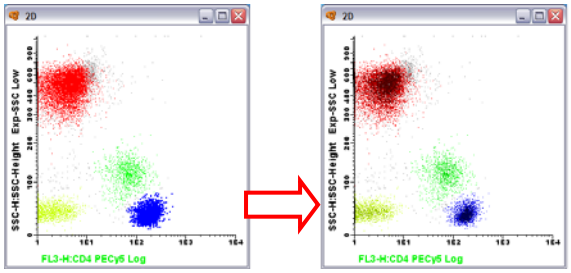
Whenever it is necessary, the user can change the 2D Plot into a density plot by only activating this function in Configure Visualization Data. The Plot will change the appearance automatically.

No density: this is the default choice. Unless the users change it, the graphs appear always without any density representation.

Total Density: This choice will consider all the events visualized to perform a density display.

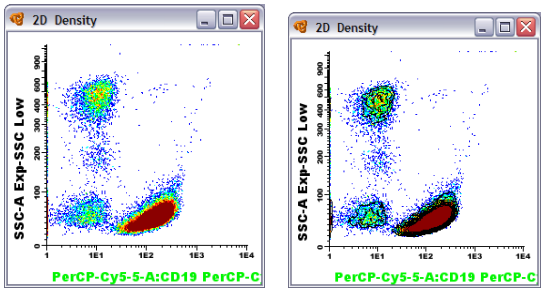


Population Density: This choice will consider the populations already classified to maintain the colors for the density representation. The density will be represented degrading the chosen color.



-Density Configuration: Density representation

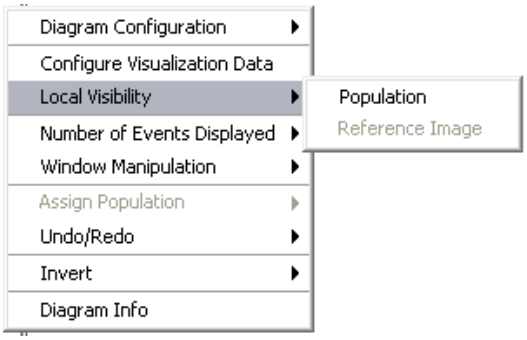
The user can represent also contour lines. In the following figure you can visualize the same diagram with total density and with total density and contour lines.



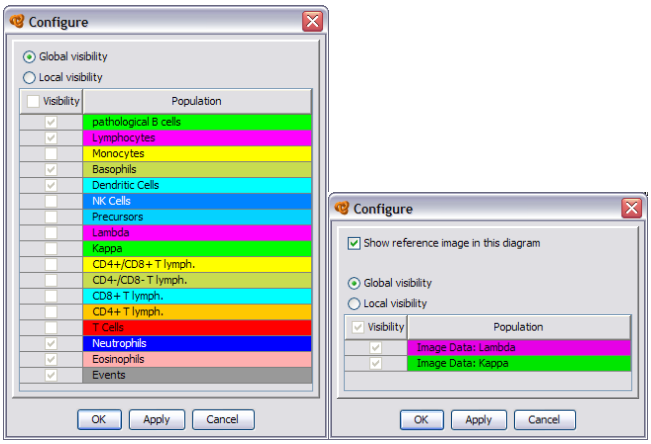
-Density increment: Using this scroll, the density of the graphs can be configured in order to choose the best contrast (increment) between the colors in it.

4.7.3. Local Visibility

Initially, the populations represented in a diagram are the ones which are selected in the **Vis column** of the Manual Analysis window. The user can go to this option to have a special visualization in one or more of the diagrams. The special visualization can apply to the population tree, and to the reference picture.



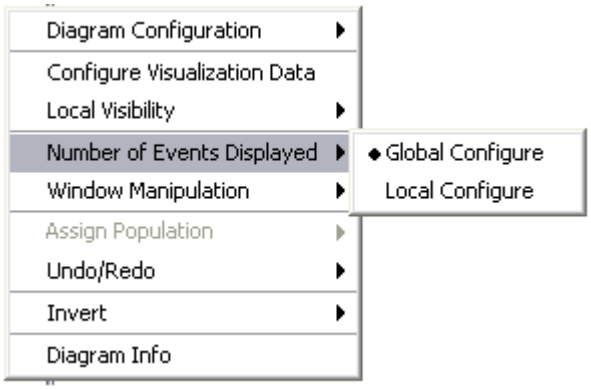
In both cases, if you need a specific visualization in a concrete diagram locally, just disable Global Visualization option, and clicking on Local Visibility you can mark the populations you want to visualize. This will apply only on that diagram.



When the local visibility is active, a warning (VIS) will appear in the frame of the graphic to remind you that you are working on special visualization. The rest of the dot plots will follow the operations you make in the Manual Analysis Window in the checkboxes VIS of the population tree, but the dot plots with special visualization will work independently.

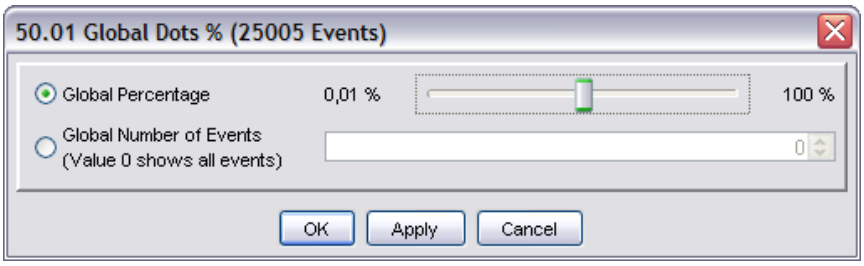
The warning message VIS will appear in the left part of the upper side bar, the same place as GATE, %, REV,...

4.7.4. Number of Events Displayed

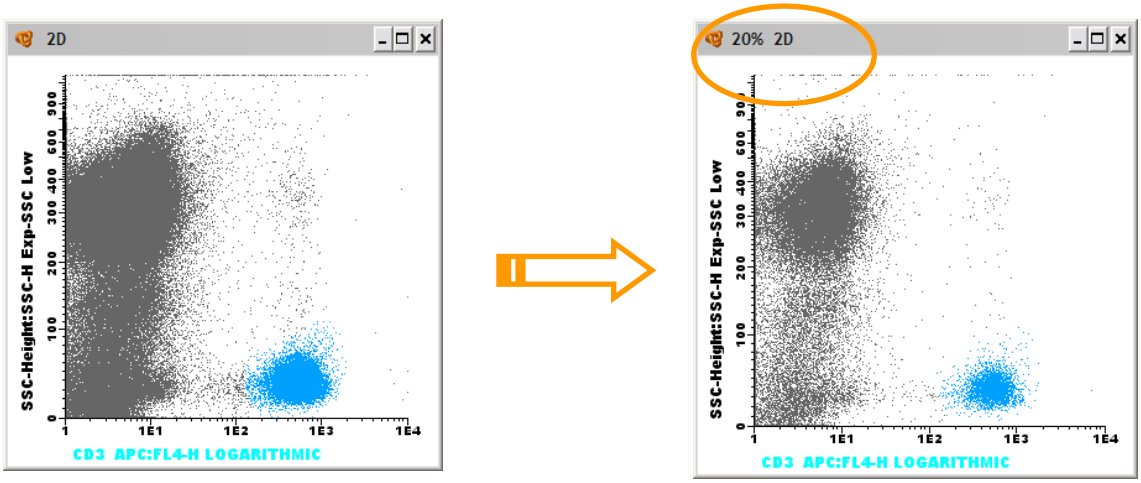


This option allows the user to reduce or increase the number of dots displayed in the dot plot. This action can be performed locally on the chosen dot plot (**Local**) or globally applied to all the diagrams (**Global**).

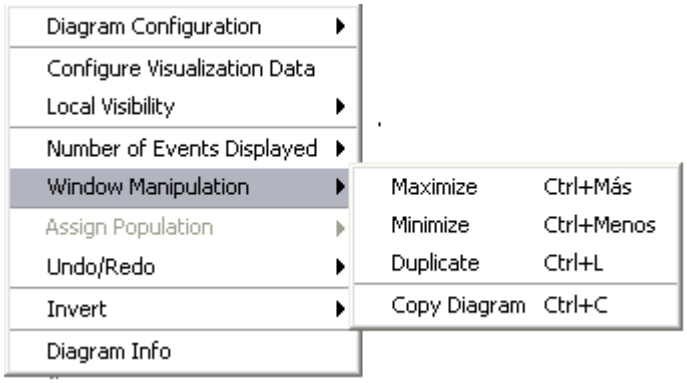
When one of these options is selected, a dialogue box appears where the user can select the percentage of dots by moving the cursor with the mouse or choosing an exact number of events to show.



Once you have selected the appropriate percentage, click OK and the graph will contain the new percentage of events, and a warning in the title of the diagram will warn you about this change.



4.7.5. Window Manipulation



4.7.5.1. Maximize and Minimize

With these commands, (**Maximize** or **Ctrl+** and **Minimize** or **Ctrl-**). the user can increase or decrease the size of the dot plot .

4.7.5.2. Duplicate Diagram

The option **Ctrl +L** creates a duplicate of the same graph with exactly the same configuration, preferences and analysis that the copied one.

4.7.5.3. Copy Diagram

The option **Ctrl+C** allows copying the diagram for a later paste in different software such as PowerPoint.

4.7.6. Assign population

This option allows the user to classify a population without clicking in the population tree. The same populations and colors defined in the tree are also present in the "*assign population*" list. Therefore, after drawing the gate, the user can right click on the diagram and go to **Assign Population**.

4.7.7. Undo/Redo

This option gives the user the possibility of cancelling or doing again the last modification in the analysis procedure. These functions undo and redo, respectively, the last operation performed or undone in any of the currently open diagrams. There is unlimited number of actions of undo or redo.

4.7.8. Invert

4.7.8.1. Invert Axes

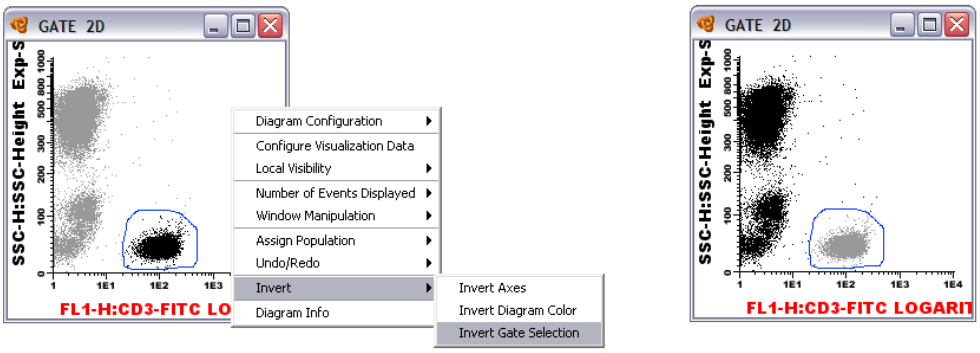
This command acts by inverting immediately the axes represented in a 2D dot plot.

4.7.8.2. Invert Diagram Color (Black/White Background Color)

This option allows the user to configure the background of the graphics, initially shown in white. Click on “black” or “white” if you prefer respectively a black or white background for presentations. This action applies only to the selected graphic. In the **Profile/Configure/Graphs** menu (4.13.5.10), the user has the option to change the default background color as a default for all the graphics.

4.7.8.3. Invert Gate Selection

It is possible to draw a region around a population and invert the gate to select all events excluded in the region. This can be done in the right click menu of the graph, and choosing “Invert” and then “Invert Gate Selection”.



4.7.9. Diagram Info

This option allows the user to obtain the information about the type of diagram and the dimensions included.

This information is especially useful in the APS view, so the user may know which parameters were relevant, being the principal components in the separation of populations. It is especially relevant for the merged data files, where the user can check which parameters are included in the data file.

4.8. Profiles

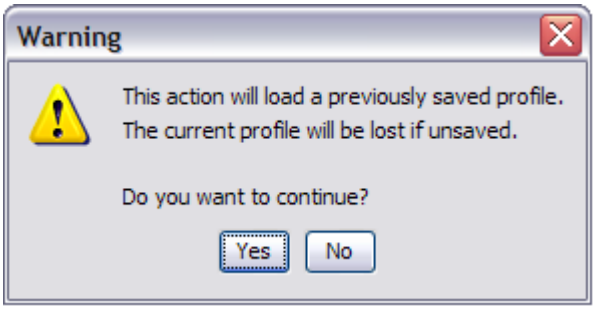
The profile is the configuration of the workspace as well as other internal configurations of the software. In this sense, the user can save different *profiles* where will define the diagrams in the screen, the population tree, the model of report, and other detailed configurations. The detailed configurations can be found on the Manual Analysis Window Menu bar, in the option Profile, Configure. In this window, different choices for changing options can be reviewed. All the changes made here will apply to the program and will be part of the profile when saved.

Save a profile

Once you have defined the diagrams, population tree, report and the other internal configurations, go to **Profile** in the Manual Analysis Window Menu Bar, and choose **Save Profile**. Automatically the software will ask you where to save it and the extension applied will be **.inp** (Infinicyt™ profile).

Open a profile

Before, or after opening a sample, you can always go to **Profile**/**Open Profile** and the software will ask the user to select the profile of interest. Then, choose Select. The software will warn that when applying a saved profile, the current one will be lost, unless you want to save it first.


Warning
This action will load a previously saved profile.
The current profile will be lost if unsaved.
Do you want to continue?
Yes
No

4.9. Reference Image

Reference image is a useful tool to compare events previously stored as a reference with a new sample that we are analyzing. In this sense, basal conditions of a sample, cells at diagnosis, or normal patterns can be saved as a reference guide to have it available whenever we want to analyze the same type of sample but in different conditions.

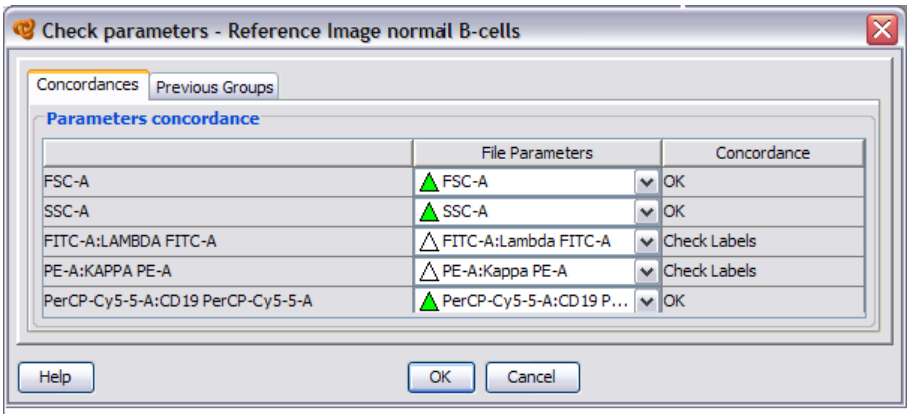
4.9.1. Save Reference Image

Any time we want to save the cells on a given dot plot as a reference, just go to **Profile**, **Save Reference Image** in the Manual Analysis Window Menu Bar, and all the events visualized will be stored in this reference image. Therefore, pay attention on saving as a reference image only the populations of interest instead the whole sample events.

4.9.2. Load Reference Image

Whenever the user is interested in recover a Reference Image to compare with the current sample, just go to **Profile/Load Reference Image** in the Manual Analysis Window Menu Bar, and choose the one you want to apply. Infinicyt™ will automatically check if the current parameters where you are applying the reference Image are the same than the parameters in which you had previously saved the Reference Image.

If there are discrepancies in the parameters, the following dialogue will be shown:

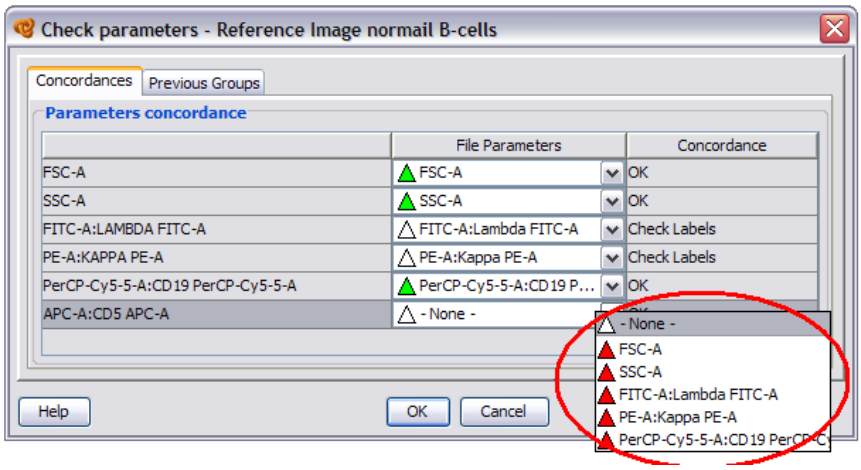


If the light is green, this parameter matches; it means that the label is exactly the same and the reference image can be applied in that parameter.

If it is white, it means that there is a quite similar parameter, but not exact (maybe typing differences) and the user should **check** in the pop-up list that this is the right one.

The user can look in the pop-up list, but some parameters will appear in red light for being not possible to establish a correspondence. If there is not parameter to correspond,

choose *None*, and the process will continue, although the reference image wont be shown in a parameter with none correspondence.



Once this step is performed the Reference Image will be shown in all the possible diagrams.

In the population tree a new label under each color will appear, because the reference image can also be shown or hidden. The statistics can also be compared, because the statistics of the reference image event are the ones that the sample had in the moment was saved.

4.10. Save, Apply and Review Analysis Strategies

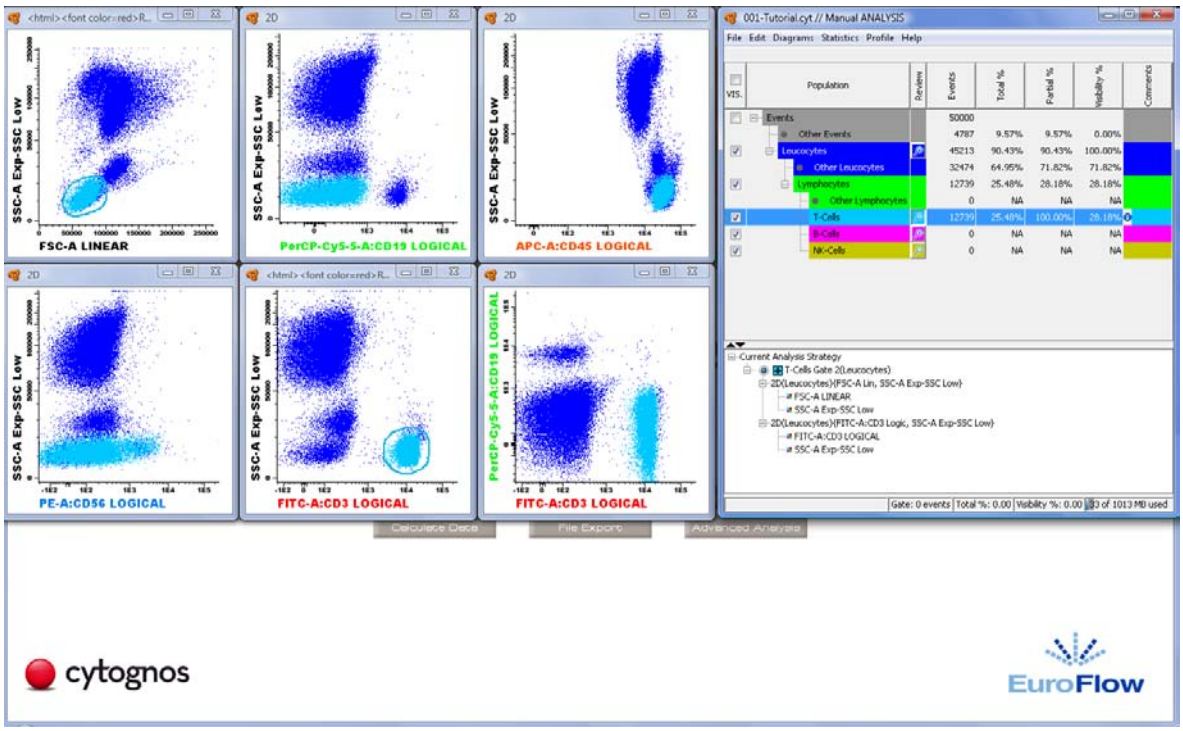
In version 1.4 the user can review the strategy of gating for each population, the user can also save a Strategy of gating and apply it over other files.

4.10.1. Review a Strategy

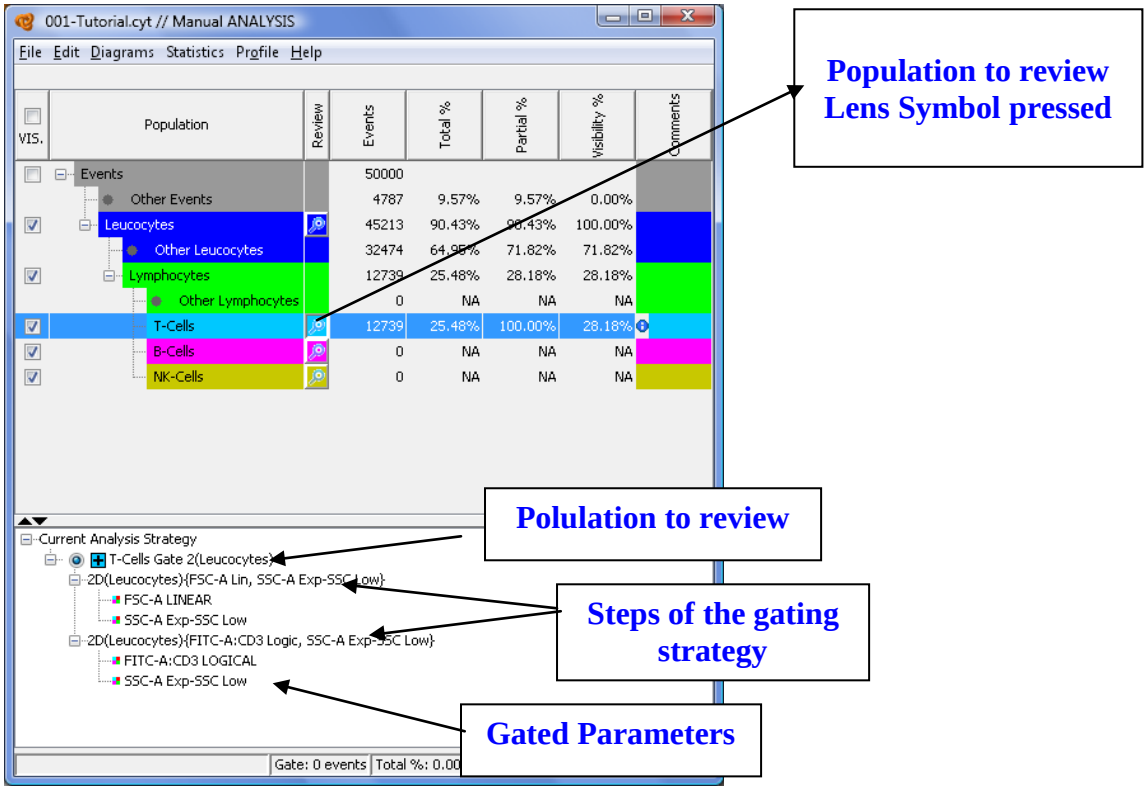
Every time you need, you can review the selection of the populations.

Go to the label of **"T-Cells"** and by right clicking select the option: Load Gates Review.

At this time a new column will be created with the symbol in all populations with events. It will appear also some diagrams showing the strategy of gating you made for characterize the populations, **"T-Cells"** in this case

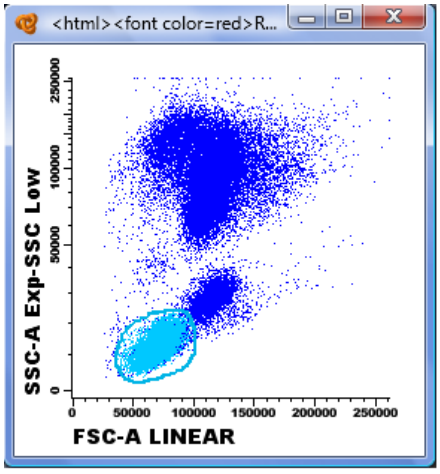


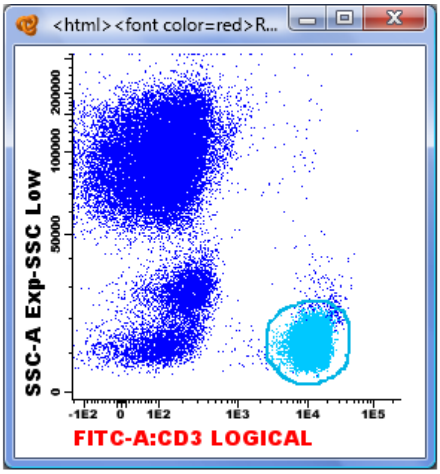
Looking in detail the Manual Analysis Window:



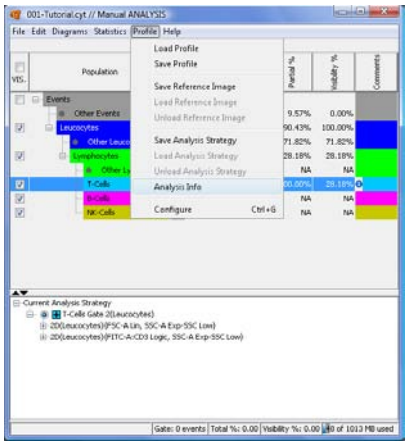
Looking at the diagrams, Infinicyt™ shows in which diagram and with which visibility we have made the gates to identify a population.Gates can be positive + to include envents n this population or negative – when the aim has been excluding events from that population.

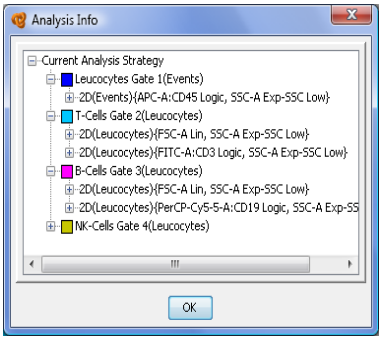
For "**T-Cells**" Infinicyt™ shows us the following diagrams:





By clicking on **Profile/Analysis Info** on the Manual Analysis Window Menu Bar we can see the whole strategy made to get the results.

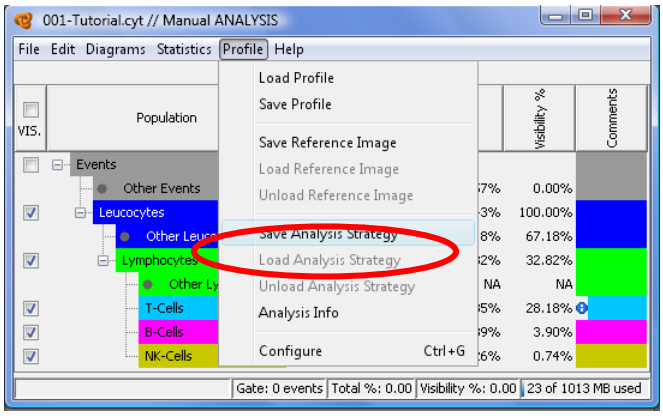




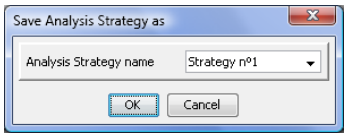
4.10.2. Save Analysis Strategies

After performing the analysis of the populations, is possible to save the gate strategy.

Go to **Profile/Save Analysis Strategy** on the Manual Analysis Window menu bar.



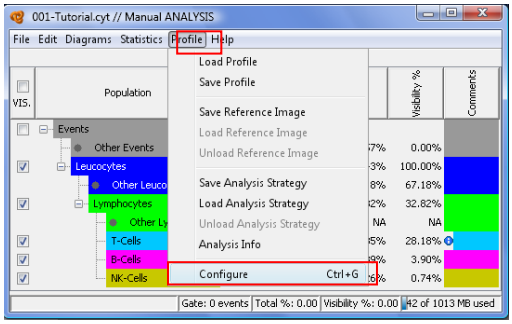
Name the strategy

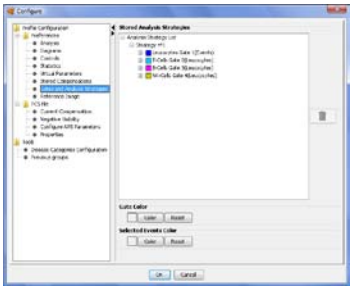


This new Strategy has been stored in the profile.

To check the strategy, go to **Profile/Configure** in the Manual Analysis Window Menu Bar, and a new window will be open**.**

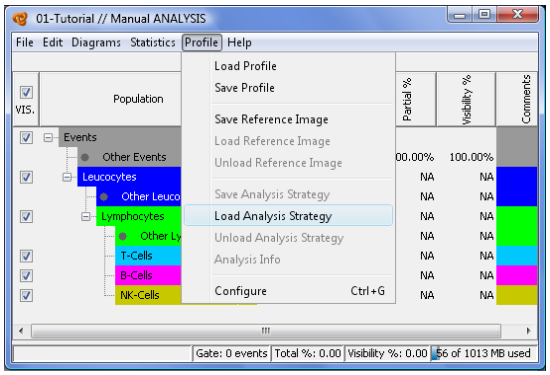
In the new window select **Gates and Analysis Strategy**.

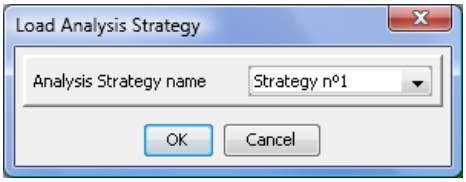




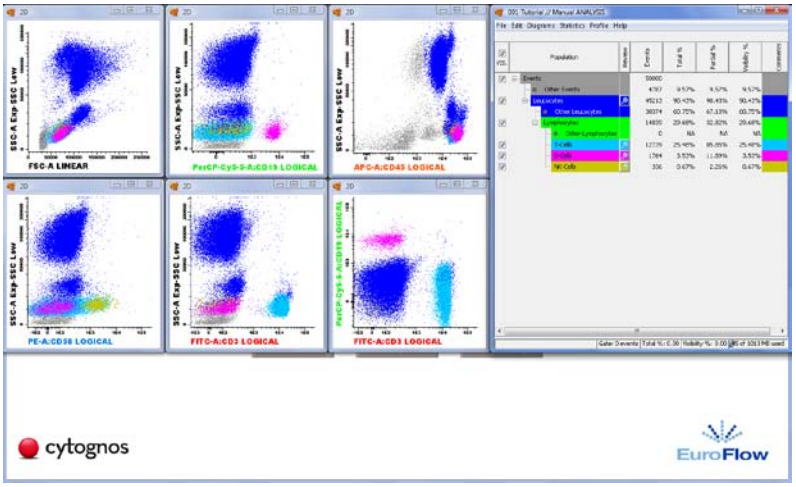
4.10.3. Apply Strategies

Open a new file and Go to **Profile/Load Analysis Strategy** in the Manual Analysis Window Menu Bar and select the previously saved strategy.





The saved analysis has been applied on the new file, just check, by clicking on the lens of each population.



4.10.4. Matching of Parameters

Infinicyt™ will only apply Saved Strategies if the parameters are coincident in both files, for example: same parameters with same writing.

In case the labels of the parameters were not exactly the same, Infinicyt™ will suggest you an option to match the parameters.

File1 where we have created the strategy.

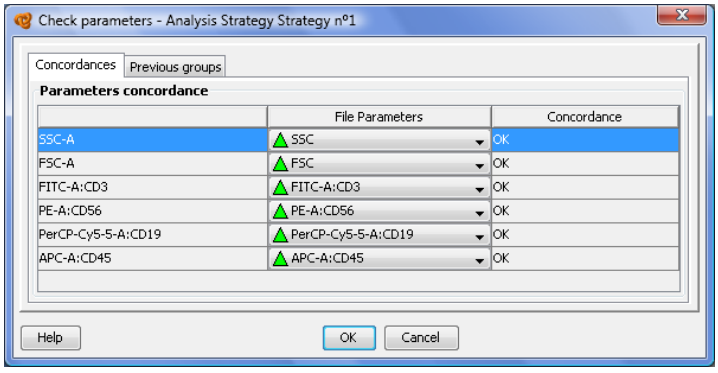
Parameters	1
FSC-A	R
SSC-A	R
FITC-A:CD3	R
PE-A:CD56	R
PerCP-Cy5-5-A:CD19	R
PE-Cy7-A	R
APC-A:CD45	R
APC-Cy7-A	R
Pacific Blue-A	R
AmCyan-A	R

File 2, the file over we apply the strategy

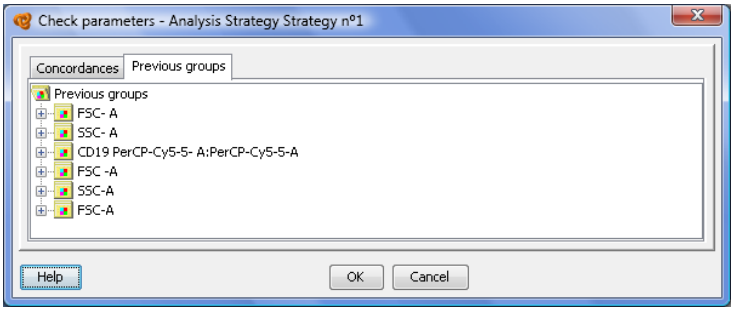
Parameters	1
FSC	R
SSC	R
FITC-A:CD3	R
PE-A:CD56	R
PerCP-Cy5-5-A:CD19	R
PE-Cy7-A	R
APC-A:CD45	R
APC-Cy7-A	R
Pacific Blue-A	R
AmCyan-A	R

Notice that FSC and SSC are not written in the same way in both files.

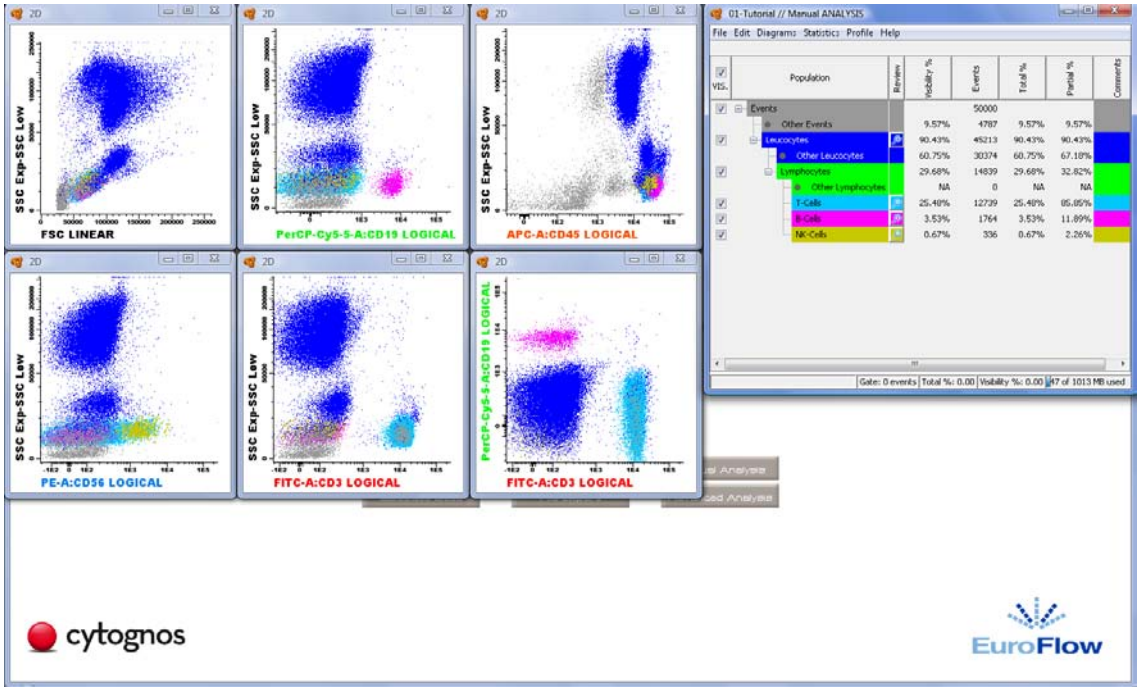
When we apply the strategy on file 2 the software shows us a box for the parameter correspondence.



Infinicyt™ keeps all correspondences and build a dictionary with every option included..

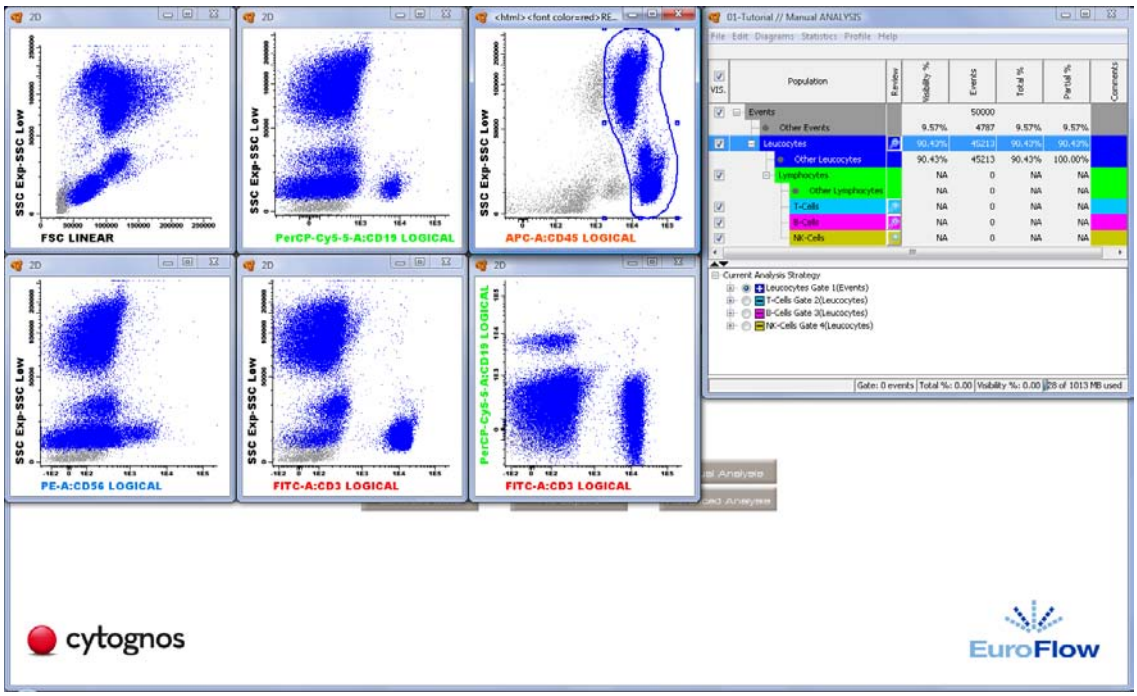


Clicking OK, the strategy will be applied in the other file.

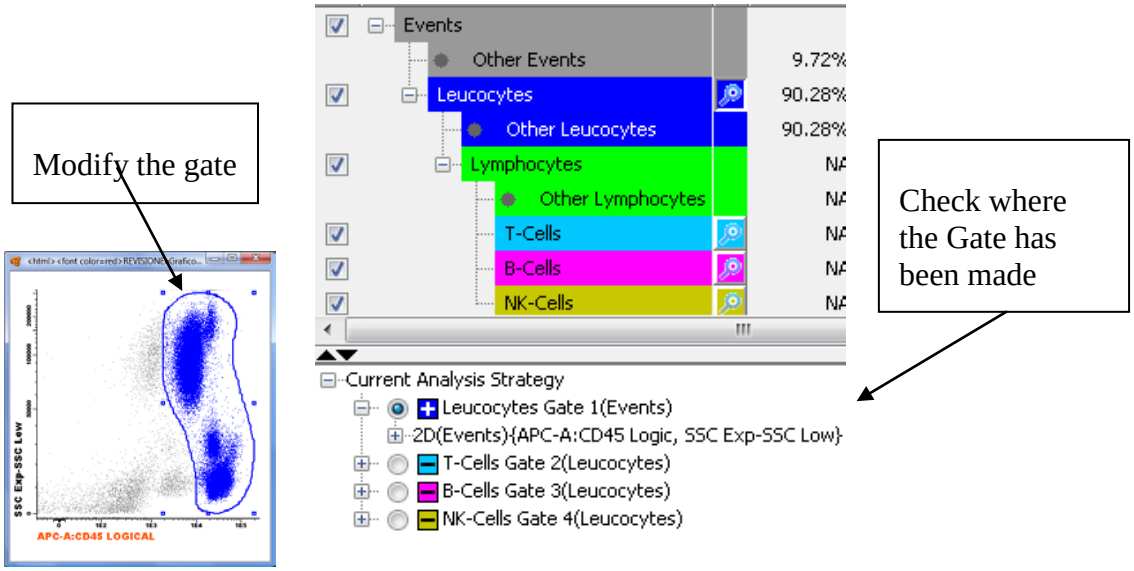


After applied, it is possible again to click on the lens of the population to Review the gates. It will appear the gate saved on the diagrams made and with the same visibility. If the gate does not fit on the new file just click on it and modify.

Review the population "**Leucocytes**":

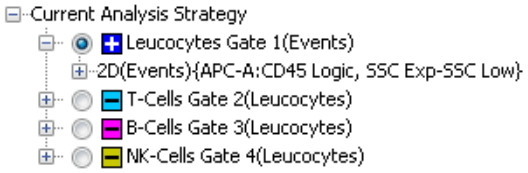


Looking in detail all information regarding the populations to review:



The + and the – means if the gates are positive or negative.

In this example, the gate for Leucocytes was positive; therefore some events were designate as Leucocytes. The negative gates indicate that some events called Leucocytes were moved to different populations, T-Cells, B-Cells and NK-Cells.



4.11. Save Analyzed files

Infinicyt™ allows saving the files already analyzed keeping the classification of the populations, the statistics…

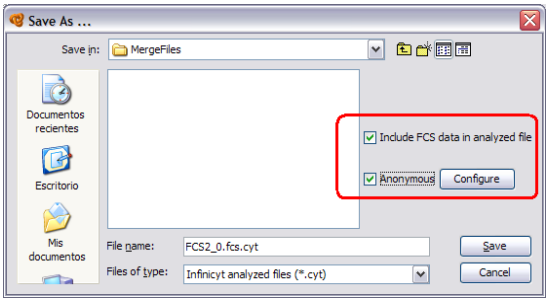
To save the analyzed sample when the analysis has finished, go to **File/Save Analyzed File** on the Manual Analysis Window Menu Bar and Infinicyt™ will immediately ask you where you want to save that file. It will be saved with the extension **.cyt**.

WARNING: In the .cyt files it is also included the profile in which it was created. Then, a way of calling a profile can also be opening a **.cyt** file that was analyzed with the profile of interest.

Notice that whenever we perform this action, there is the opportunity of:

Include the FCS data in the Analyzed file: as a default the new .cyt file will include also the raw data. If the user unclicks this option, only the data analyzed will be saved. The advantage is that this file is much lighter in memory but, if you want to open this .cyt file the software will ask you to open also the raw FCS file.

Anonimize the file. This can be done selecting the option anonymous in the saving window. The fields not matched will be excluded from the file to save.

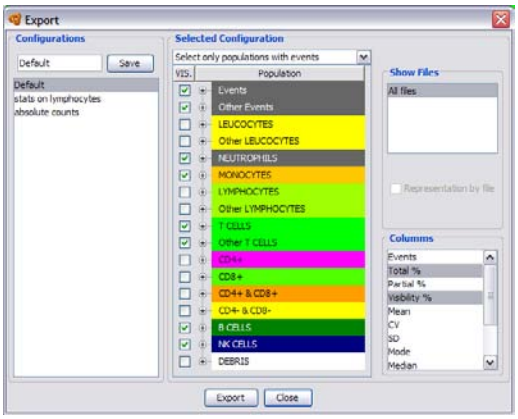


4.12. Export statistics from Manual Analysis

The export option from the Manual Analysis Window Menu Bar (**Statistics** / **Export Statistics**) is a tool to export to other platforms the data of the analysis.

When the export option is open, this window will appear where and user can configure which population, which parameters and which value of each to export.

It is possible to **Save** different configurations of statistics to avoid repeating the selection of fields every time.

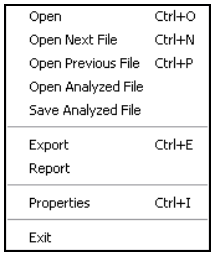


Then, clicking on **Export** the software will ask where to save this new file with the statistic data. The proposed extension for this statistic file will be **.csv** (coma separated values) which can be read for any worksheet, database or word-processing third-party tool.

4.13. General description of the analysis menu

4.13.1. File

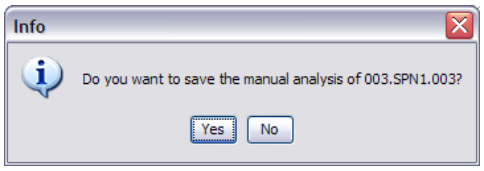
In this section we described all the options that can be found on the File Menu in the Manual Analysis Window Menu Bar



4.13.1.1. Open

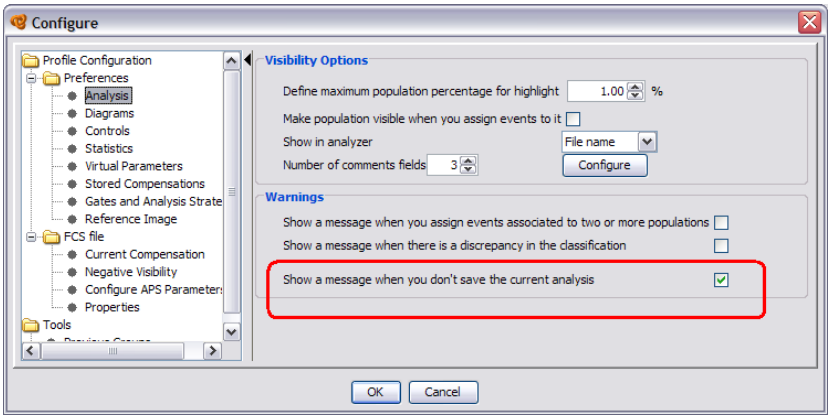
When choosing this option (**Ctrl+O**), a File selection Window will appear where the user can select the FCS file to analyze. Once this is made, the user must click the "Select" button of this screen to load the file and see the results. By clicking on the "Cancel" button, the selection screen disappears. The FCS file may be in any folder of the computer where the user has reading permission.

If the user had already opened a file, the software will ask if he wants to save the manual analysis performed in the previous file, before continuing opening a new file.



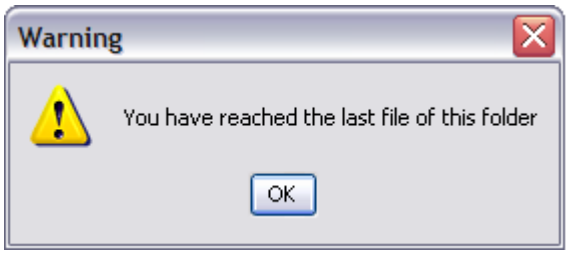
This warning can always be activated reminding to save the analyzed files when finishing. This message can be activated in Profile/Configure/Analysis of the In this section we described all the options that can be found on the File Menu in the Manual Analysis Window Menu Bar.

.If this option is deactivated, the files analyzed won't be saved unless you do it through the option *File/ Save Analyzed File* in the menu.



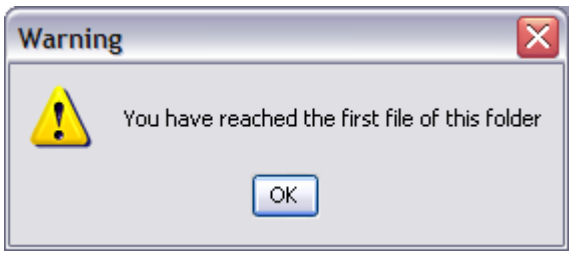
4.13.1.2. Open Next File

Choosing this option (**Ctrl+N**), the software will automatically load the next FCS file stored in the same folder as the previous file until you reach the last file of the folder. When this occurs, and the user tries to open the next one the following message appears:



4.13.1.3. Open Previous File

Choosing this option **(Ctrl+P)** the software will open the previous FCS file in the folder until it reaches the first one, and the following message will appear.

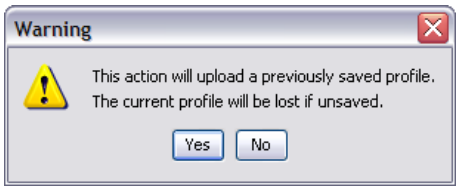


4.13.1.4. Open Analyzed File

As described on chapter 4.11, Infinicyt™ software allows saving the files analyzed, containing the information of the population tree classification, statistics, graphics, report…, and store them in the hard disk. These files will be stored as a **.cyt** extension.

Whenever the user wants to open a file already analyzed, then the .**cyt** file must be opened from the option **Open Analyzed File**.

The software will warn you that it will upload a previously saved profile. This occurs because the software stores, together with the analyzed file, the Diagrams, Population Tree, Report and settings used in that previous analysis, which it means a profile.



4.13.1.5. Save Analyzed File

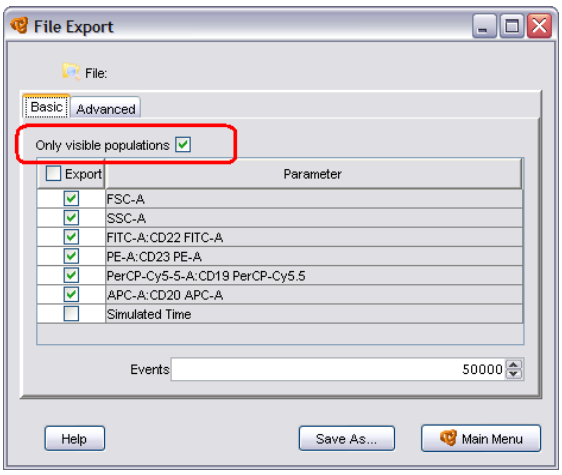
This option is used whenever the user wants to store the analysis of a file. The software will ask the directory where to save it and the file will be saved as **.cyt**. The original data file will remain the same in the corresponding folder, because the **.cyt** file does not affect to the original one.

A .**cyt** file will include the analysis of populations with the corresponding Population Tree, the Diagrams, the Statistics, the Report….and all the information contained on the profile used to analyze it.

4.13.1.6. Export

When this button is clicked (**Ctrl+E**), the program goes to the *File Export* Option, and in this case, the file to export will be the one currently opened with the analyzer.

For that reason an additional option is included in this window and it is used to "export only visible populations". With this tool, it is possible to export gated events or some specific population.



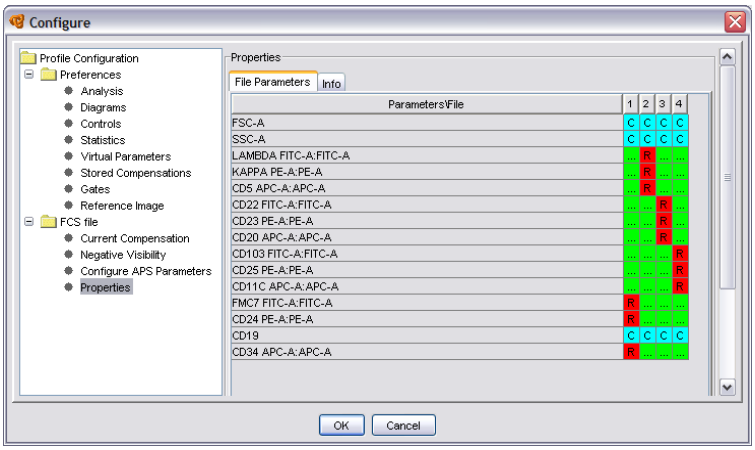
See Export chapter 9 for more information.

4.13.1.7. Report

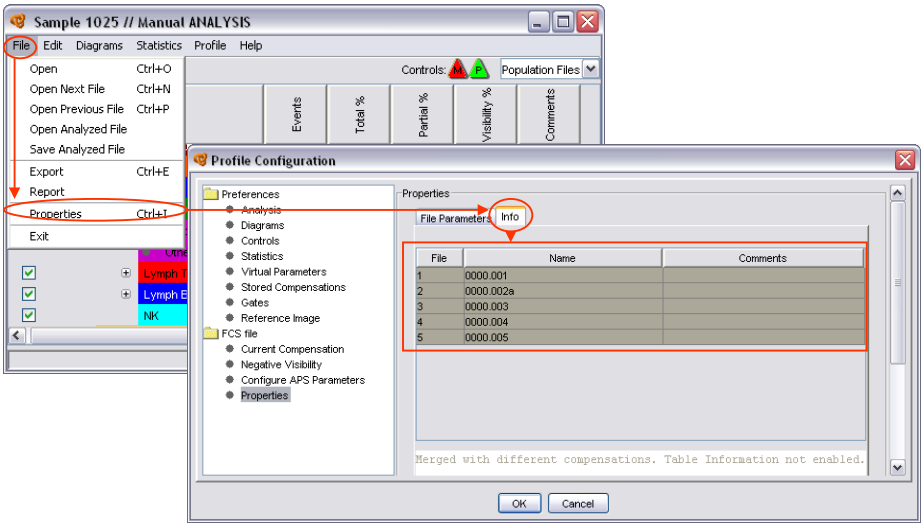
Through this option the user may do printable reports. Initially, a blank page appears and the user includes different elements, such as graphics, statistics and comments of interest. See chapter 5 "**Printed Reports"** for more detailed information about this issue. The report will be saved as part of the profile, and will be automatically uploaded when you open new data files.

4.13.1.8. Properties

With this command (**Ctrl+I**), we open the *Profile Configuration*/*Properties* window of the data file, to check all the parameters measured. This is especially useful when working with merged data files (chapter 6) because in this window we will always have available the matrix with the information for all the parameters and files.



The option **Info** (in the second tab of this window) will show us any comment written on the original data file during the acquisition. Even in a merged data file, you can visualize also the names of the files.

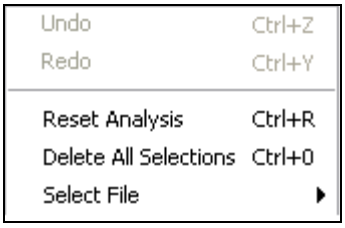


4.13.1.9. Exit

When choosing this option, the program will exit the *Manual Analysis* mode, closing all the windows associated with this process, including the Manual Analysis Window and all the Diagrams. The *Manual Analysis* session can also be finished by closing its *Analysis Window*, with the same result.

4.13.2. Edit

In this section we described all the options that can be found on the Edit Menu in the Manual Analysis Window Menu Bar



4.13.2.1. Undo / Redo

These options apply exclusively to the operations performed during the analysis. Other actions regarding configuration of the diagrams or application settings cannot be undone.

"Undo…" (Ctrl+Z): By using the first of these options, it is possible to undo the last operation performed in the event selection procedure, whether this operation was a drawing step or a modification of the classification of events, or any of the operations correcting the selection procedure.

"Redo…" (Ctrl+Y): Performs the inverse operation, i.e., it redoes any operation undone by the "Undo" function.

Both options become active only when there are operations that can be undone or redone, and in this case the text appearing in both buttons includes the description of the type of operation that will be undone or redone by using the corresponding option.

These *Undo/Redo* options of this menu are the same options we can find if we click in the middle of the plot with the right button of the mouse, opening a pop-up window, which includes these options among many others referred to the diagram. There is an unlimited number of undo and redo actions in memory.

4.13.2.2. Reset analysis

Choose this option (**Ctrl+R**) to clear all the analysis gates and go back to the initial situation with all the events classified as “Events”.

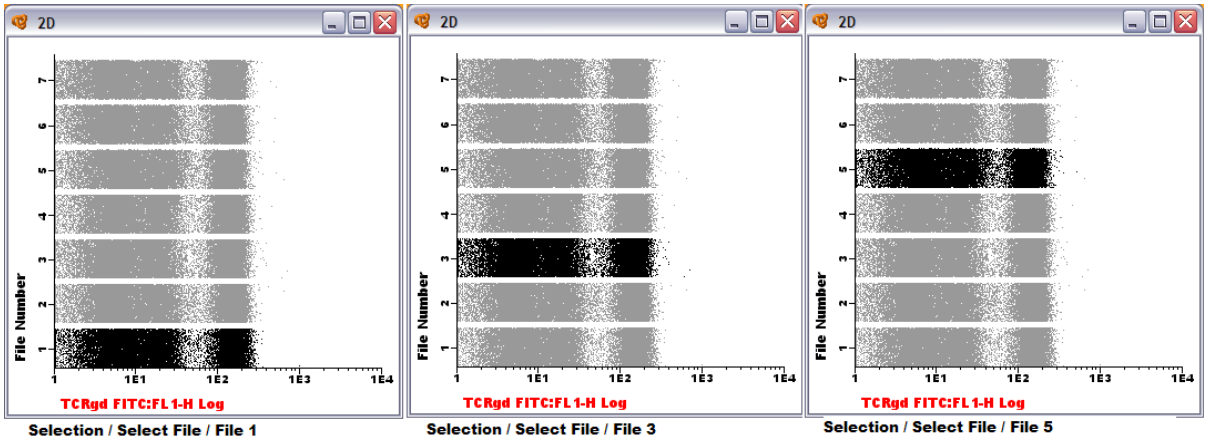
WARNING: Reset analysis will apply to clear all colors in the analysis, but not to any change made on the options concerning the profile.

4.13.2.3. Delete All Selections

Choose this option **(Ctrl+0)** to delete the consecutive regions performed in several diagrams before classifying a population.

4.13.2.4. Select File

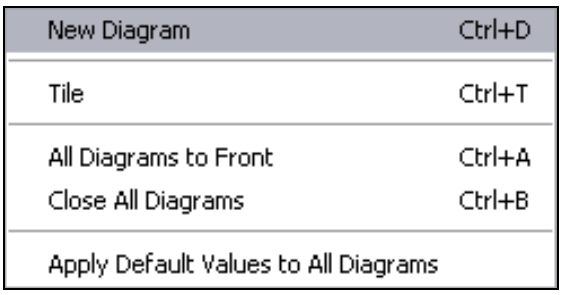
This option applies only when working with merged files (Chapter 6). Through this option, the user can select (gate) all the events corresponding originally to file number 1 (or Ctrl+1), file number 2 (or Ctrl+2)….etc, without having to draw the region with the mouse.



WARNING: Any other selection previously done without assignation of color, will be lost if we perform this type of selection of the whole tube.

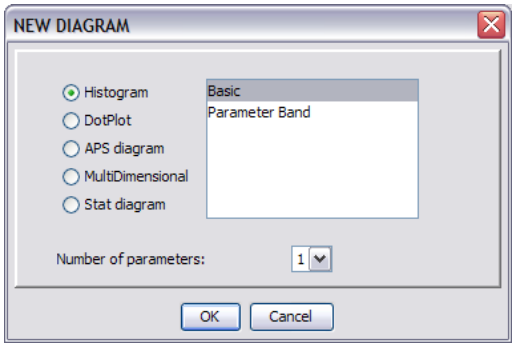
4.13.3. Diagrams

In this section we described all the options that can be found on the Diagram Menu in the Manual Analysis Window Menu Bar



4.13.3.1. New diagram

When clicking this option (**Ctrl+D**), the following dialogue box appears:



The user may select among different kind of diagrams (see details in chapter 4.6):

Histogram

DotPlot

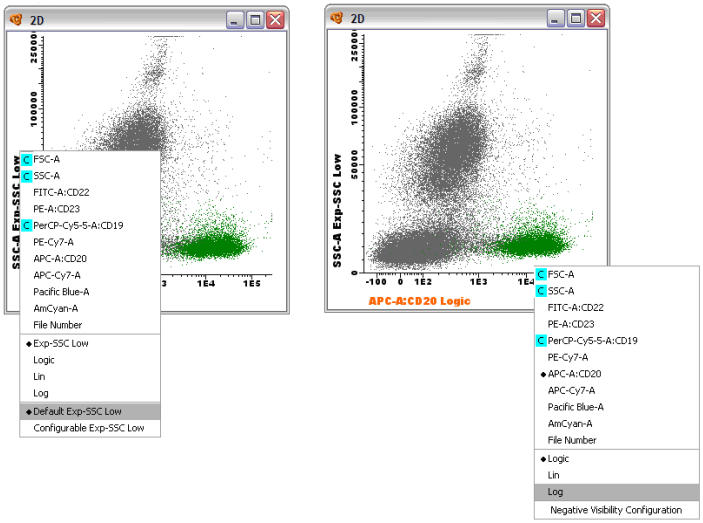
Automatic Population Separator Diagram (APS)

MultiDimensional Graphics, where the user may select the number of dimensions.

Statistical Diagram, which includes Box Plots.

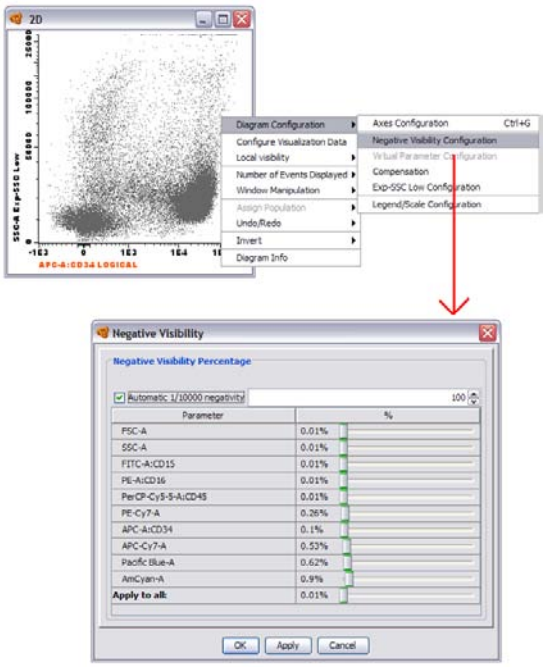
Once the user selects the diagram, the appropriate parameters can be chosen by clicking on the axes of the figures only in case of DotPlots. The configuration of the axes is also modifiable through the right click diagram menu (**Diagram Configuration/Axes Configuration**). (see 4.7.1: *Diagrams Configuration*).

When you click the axes to select the appropriate parameter, you can select both the parameter (in the upper part of the pop-up list) and the scale (lower part of the pop-up list). When you are analyzing a merged file the common parameters will appear with a C in the list of parameters.



Type of scale representation.Log and Logical Scales. Negative visibility.

The selection of the parameters scale can show the information in linear scale (**Lin**), in Log scale (**Log**) or with logical transformation (**Logic**). Logical scale shows negative values, if they exist, as a result of compensation. The part of the negative scale that we want to visualize can be configured in the right click menu of the diagram, in the option *Diagram Configuration/ Negative visibility Configuration.*

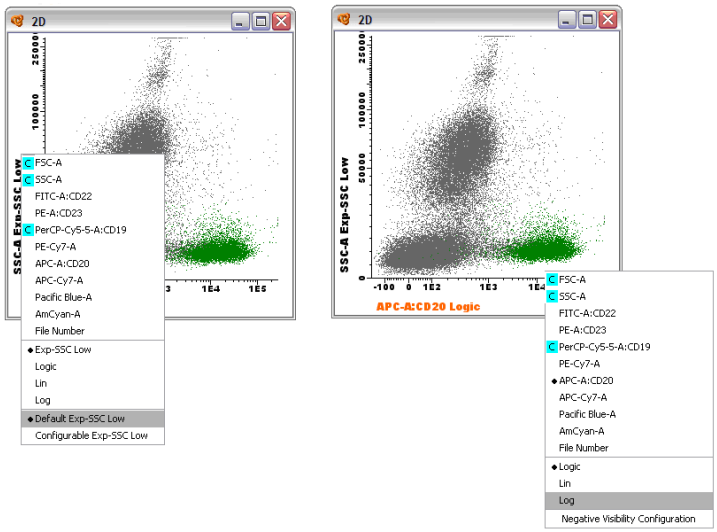


When a logical transformation is represented, the user has the choice of configuring the negative part of the scale for a better visualization with the Negative Visibility Configuration. See detailed information on section 4.7.1.2 and also in the Manual Analysis Window Menu Bar in Profile/Configure/Negative Visibility on chapter 4.13.5.10.

Side Scatter transformation

Side Scatter may also be represented with a mathematic transformation to expand the values of the lower part of the scale for a better visualization of the populations (Exp-SSC-Low).

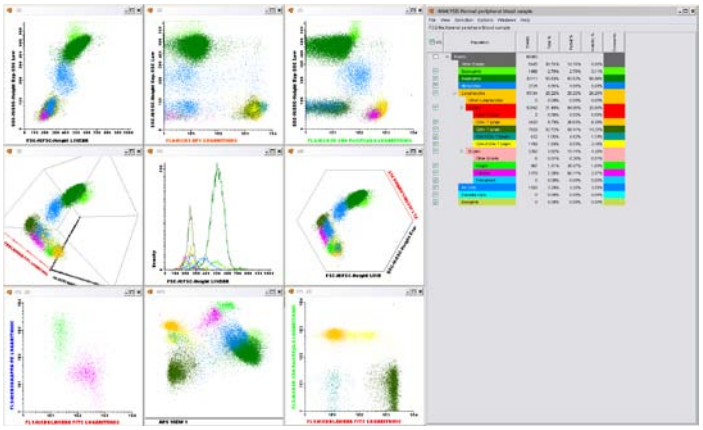
Exp-SSC-Low transformation can be used with a default transformation or it can even be configured by the user. See section 4.7.1.5.



4.13.3.2. Tile

The diagrams will resize and the program will rearrange them to cover the whole screen of the computer. In the menu Diagrams there is a short command for this option (**Ctrl+T**).

If there is no diagram open, Tile function will open several plots displaying all the different combinations of parameters.



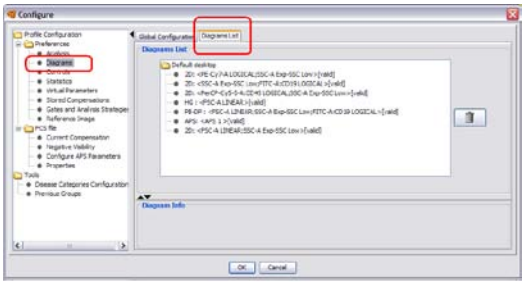
4.13.3.3. All Diagrams to Front

In case the plots are hidden behind the *Manual Analysis Window*, with this command (or **Ctrl+A**) they will be brought to the front. In case the user wants to maximize the *Analysis Window* when it has been minimized, just double-click in the middle of any diagram.

4.13.3.4. Close All Diagrams

Choose this option (or **Ctrl+B**) to remove all the windows shown in the screen if you want to create new different graphics. The diagrams which are part of the profile are listed in the following box: **Profile /Configure / Diagrams/Diagrams List** from the Manual Analysis Window Menu Bar. If any diagram is closed in the analysis will remain in the list and will be automatically opened when their parameters appear in a new file opened.

The only way to delete a diagram from a profile is from this window" Diagram List" (select the diagram and click trash), or with Ctrl+B (close all diagrams) which will delete all the current diagrams in the profile.

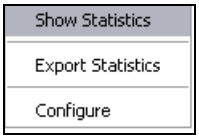


4.13.3.5. Apply Default Values to All diagrams

Whenever we resize or change the appearance of the graphs, we can always return to the saved default settings, clicking this option. Information about default values is available on Profile/Configure/Graphs.

4.13.4. Statistics

In this section we described all the options that can be found on the Statistics Menu in the Manual Analysis Window Menu Bar



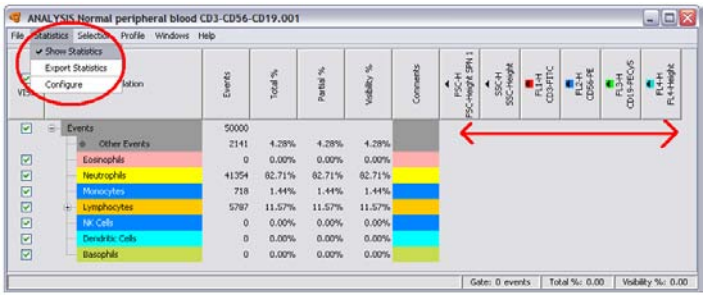
These options refer to the visualization and configuration of the main statistical data of the different populations. For each analyzed population, the program always displays some default information about its relative numbers with reference to the total cells or to partial populations, which can be shown in the *Manual Analysis Window*.

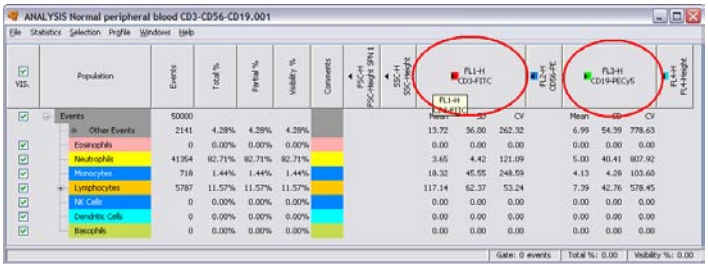
4.13.4.1. Show Statistics

The analysis window shows by default, information about the events number and percentages related to total cells or partial populations. Apart from this basic information the user can also obtain statistic values of the populations.

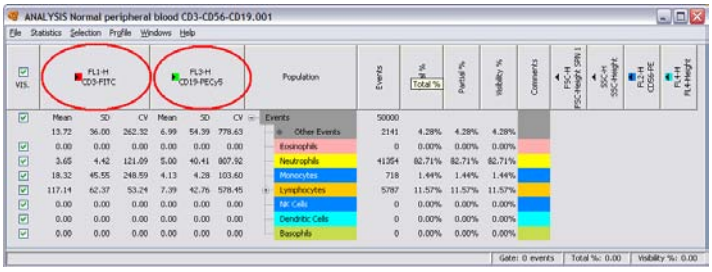
The option **Show Statistics** generates more columns, one per each parameter. Each column corresponds to the statistics for each parameter. The columns can be closed (◄), but clicking on them they are opened (►) to visualize the data so the user only has to keep open the columns needed. Then it will appear by default the Mean, SD and CV for each population. To obtain more statistic functions, they need to be configured in the profile. See chapter 4.13.5.10 to see how to configure new statistical functions.

In the **Statistics** option of the menu, you can visualize the statistics by having this option **Show Statistics** activated (√). The new columns will appear in the right part of the population window.





The information columns can be moved by click and dragging the column right or left.



WARNING: Remember that for a faster analysis, it is recommended to have the statistic view option **deactivated** in the **view** menu until you need it.

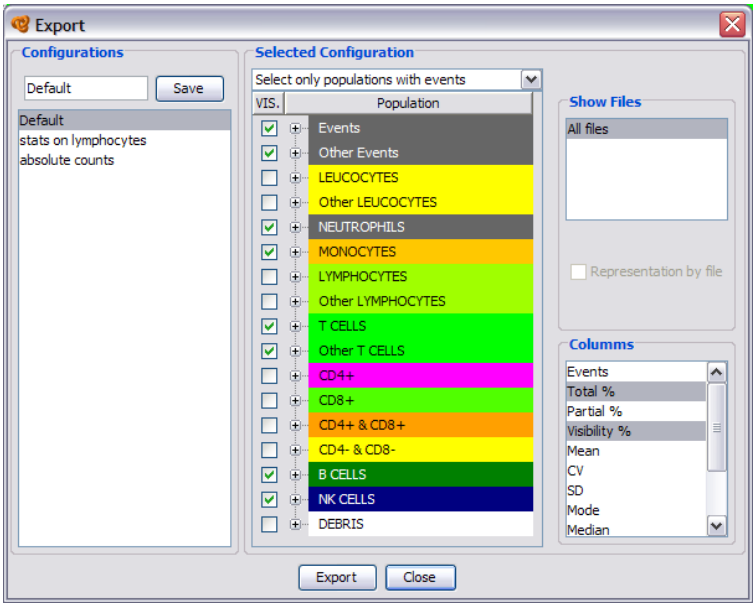
All these statistics can be shown on the report. You can build a report that always shows the statistics of interest, or you can export them to **.csv** files as described in next chapter.

4.13.4.2. Export Statistics

The export option from the Manual Analysis Window Menu Bar (**Statistics** / **Export Statistics**) is a tool to export to other platforms the data of the analysis.

When the export option is opened, this window will appear where and user can configure which population, which parameters and which value of each to export.

It is possible to **Save** different configurations of statistics to avoid repeating the selection of fields every time the user opens this tool.



Then, clicking on **Export** the software will ask where to save this new file with the statistic data. The proposed extension for this statistic file will be .csv (coma separated

values) which can be read for any worksheet, database or word-processing third-party tool.

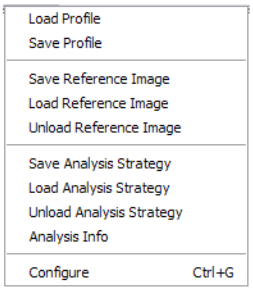
To choose the character separator in the CSV files, or the number of decimal places, go to Profile/Configure/Statistics/Format. (See chapter 4.13.5.10).

4.13.4.3. Configure

This option opens the Profile Configuration/Statistics window. In this window, the user may choose which statistical data will be shown on the analysis window, and may create new functions or absolute counts. See on chapter 4.13.5.10 Profile/ Configure/Statistics, the tools included on this window.

4.13.5. Profile

In this section we described all the options that can be found on the Profile Menu in the Manual Analysis Window Menu Bar.



A profile is all the information about the software configuration (as explained in detail on section 4.8). It is possible to create different profiles for different studies, so the user can always work with the same Graphs, Population Tree, Controls, Strategies, Statistics, Report, etc, with the same type of study.

4.13.5.1. Load Profile

Through this option, the user may open a previously saved profile with Diagrams, Population Tree, Configure options and Report. Depending on the type of study, it can be useful to work with specific diagrams and configuration. The profiles are saved in **.inp** format (Infinicyt™ Profile) in the directory chosen by the user.

4.13.5.2. Save Profile

If the user wants to save the profile of an analysis, with the Diagrams, Population Tree, Configuration and Report options, click this option and the software will open a dialogue box that asks you to choose a directory to save it. It will be saved with a **.inp** extension.

4.13.5.3. Save Reference Image

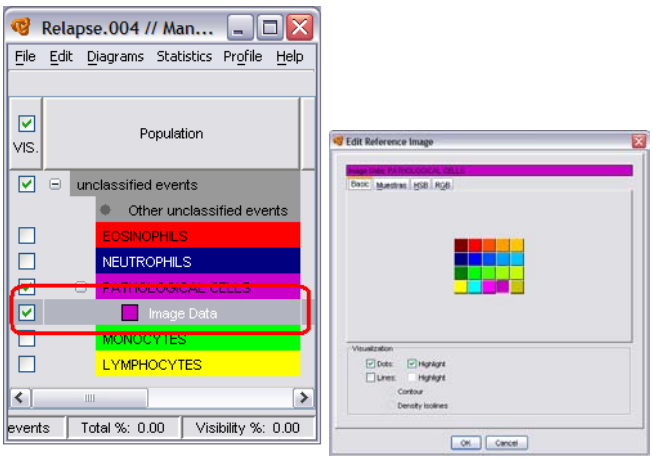
It consist on a tool to save pictures of the diagrams which can be used as a reference in similar data files to analyze. This reference pictures can appear in the same dot plot you are analyzing so the user can always have the *Reference Image* to compare (4.9).

To create a *Reference Image* you have to consider that the data file of reference and the data file you will compare later, need to have exactly the same parameters acquired. Typing mistakes can be corrected if the labels don't match when applying the Image. Once this is checked, the user must analyze the file which will be the Reference one and show in the population tree only the populations of interest. Then, in the Profile, you only have to choose "Save Reference Image" and give a name to that group of events. It is possible to save as many different Reference Images as desired.

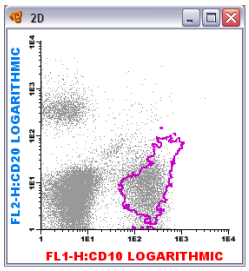
4.13.5.4. Load Reference Image

If you have previously saved a group of reference events, we can load them in the diagrams with this option: When we click Load Reference Image, we will be able to choose among the different groups we have previously saved in this profile.

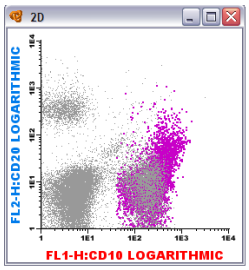
The reference events will appear in a new label with the same name and we can hide or show them clicking on the VIS box.



With a right click on the new label the user can edit the appearance of these reference events. For example, they can be displayed as highlight dots, a contour line, etc.



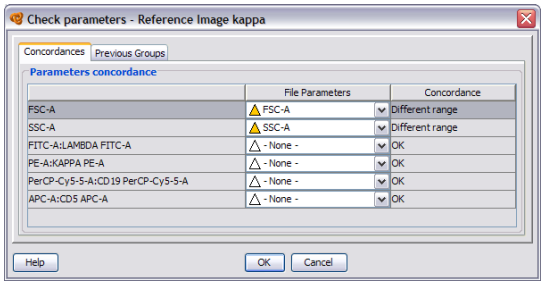
Reference Image with Lines



Reference Image with Dots

4.13.5.5. Unload Reference Image

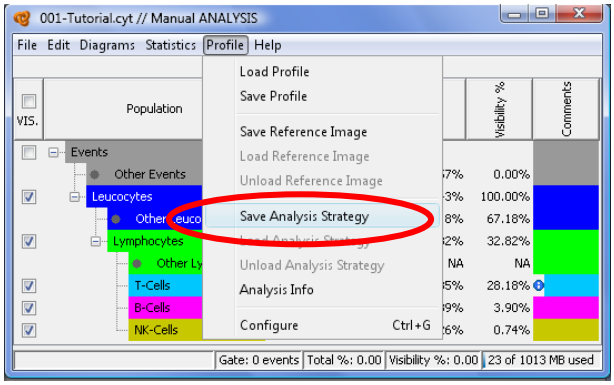
Click this option to unload the reference events. If you don't unload a reference image, and you try to analyze a different sample with other parameters, in which you can not load the same reference image, the software will try to load it and if the parameters don't match it will show you the a warning, for example like the following figure, where none of the parameters find a equivalent in the new file to load the Reference Image. If you click OK, no Reference Image will be loaded:



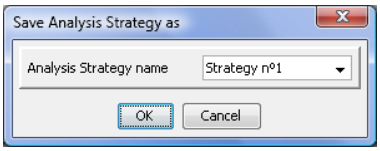
WARNING: It is important to unload the Reference Image or Strategies when you finish the analysis. Then, go to Profile/Unload Reference Image in the Manual Analysis Window Menu Bar to continue the analysis of the different sample with new parameters.

4.13.5.6. Save Analysis Strategy

After performing the analysis of the populations, is possible to save the gate strategy. For detailed information on this procedure go to chapter 4.10 (How to save Analysis Strategies). Go to **Profile/Save Analysis Strategy** on the Manual Analysis Window menu bar.



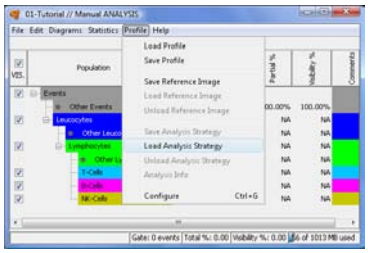
Name the strategy

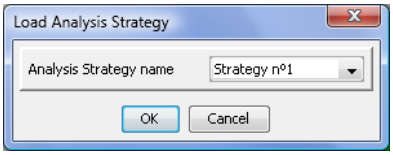


This new Strategy will be stored in the profile.

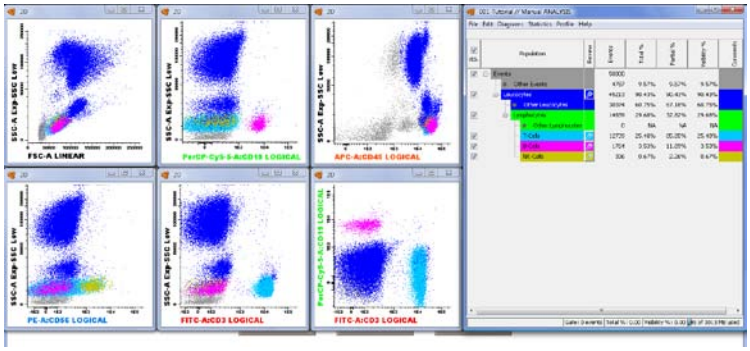
4.13.5.7. Load Analysis Strategy

To Apply Strategies, Open a new file and Go to **Profile/Load Analysis Strategy** in the Manual Analysis Window Menu Bar and select any of the saved strategies in the profile.





The analysis has been applied on the new file, click on the lens of each population to review them.

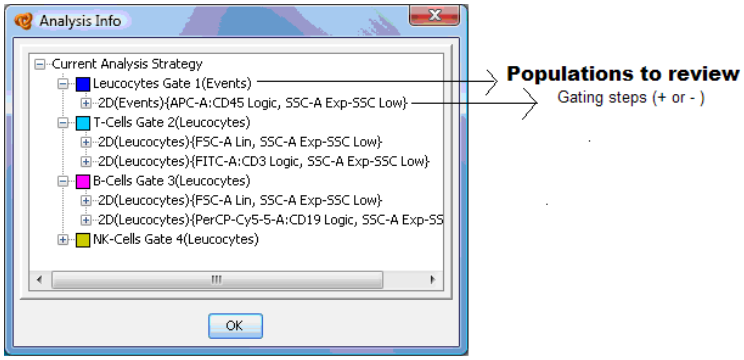


4.13.5.8. Unload Analysis Strategy

The same as with the Reference Image, it is recommended to upload the Strategy when finishing our process, in the Manual Analysis Window Menu Bar in the option Profile/Unload Analysis Strategy. Otherwise, the software will try to apply it and the parameters won't match, obtaining the warning message of Parameters Concordance.

4.13.5.9. Analysis Info

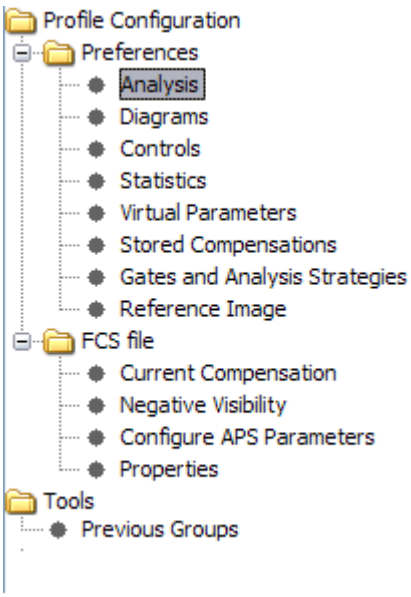
In this option the user can see all the steps of the analysis strategy



With the different colors we can see the populations analyzed, and for each of them, the different steps performed to analyze them. The steps can be positive (+) if gated events were included, or negative (-) if gated events were excluded.

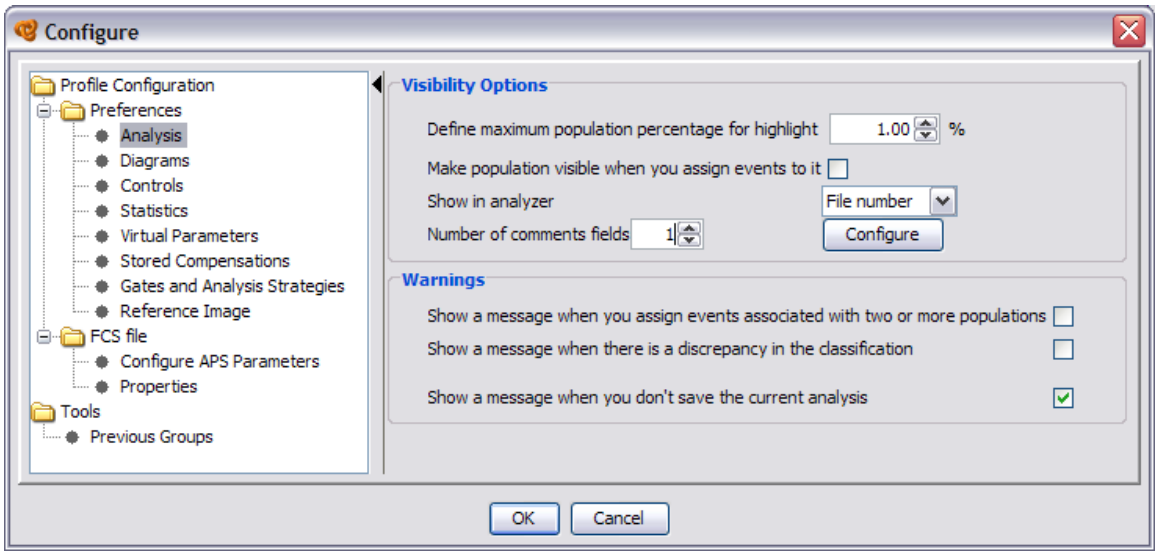
4.13.5.10. Configure

In this chapter we will review all the elements that are part of the Profile so the user may decide how to personalize a profile with the different internal parameters.



ANALYSIS

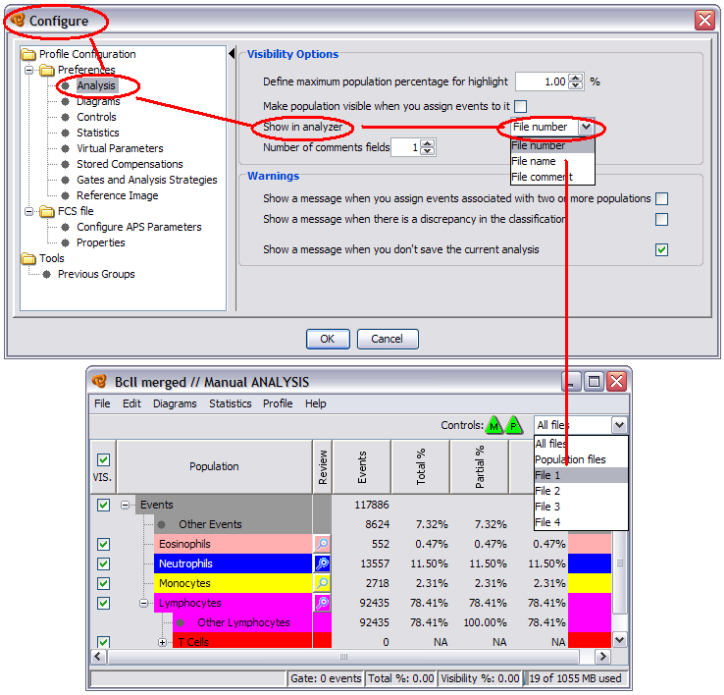
In this option the user can change some *Visibility Options* and *Warnings* during the analysis.



-To automatically activate highlighting in any defined population, the user can ***Define the maximum population percentage*** in which any population with lower percentage of events will be shown in highlight. Default value is less than 1%.

-The user can configure a default option to *always* ***make populations visible*** each time you **assign events to it**. By default, it is deactivated.

-***Show in analyzer*** will be used to choose how to identify the different tubes merged in a file.



-***Number of comments fields***. As a default the analyzer will show only 1 column for comments about the different parameters of the sample. In case you need to have more that one column for comments you can choose the number of them and their names through this option.

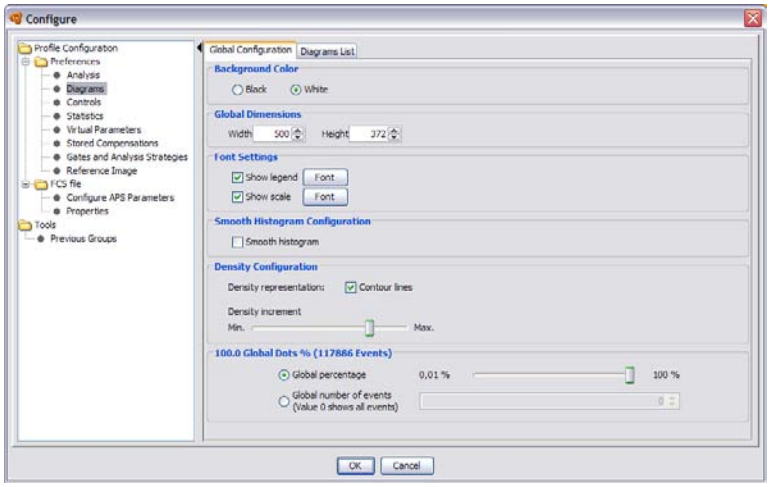
-***Show a message*** during the analysis each time the user selects ***events associated to two or more populations*** and assigns them to a new one.

-***Show a message*** during the analysis each time the user selects ***events whose populations do not derive*** *in the Population Tree* ***from a previously selected population***.

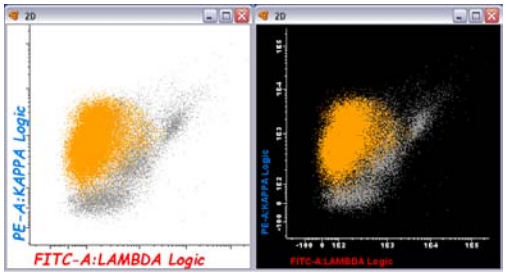
-***Show a message*** if you want that Infinicyt™ warnings you when you change to another sample ***without saving analysis***.

DIAGRAMS

-Infinicyt™ graphs can be customized by the user, as part of the profile, regarding background color, size, and font settings. Some default values are shown in this option so the user can change the appearance for each new graph.



Some changes in the Profile Configuration of the Graph may generate a totally different display of the same diagram.



Changes in this part of the profile will apply to all the graphs. If the user wants to change the appearance in only one of he diagrams, it is possible to do it one by one in the graph right click menu (see chapter 4.7.1, Diagram Configuration).

The different options to configure the diagrams in the profile are:

-Background Color: black or white is available.

-Global Dimensions of the diagrams

-Font Settings for legends and scales

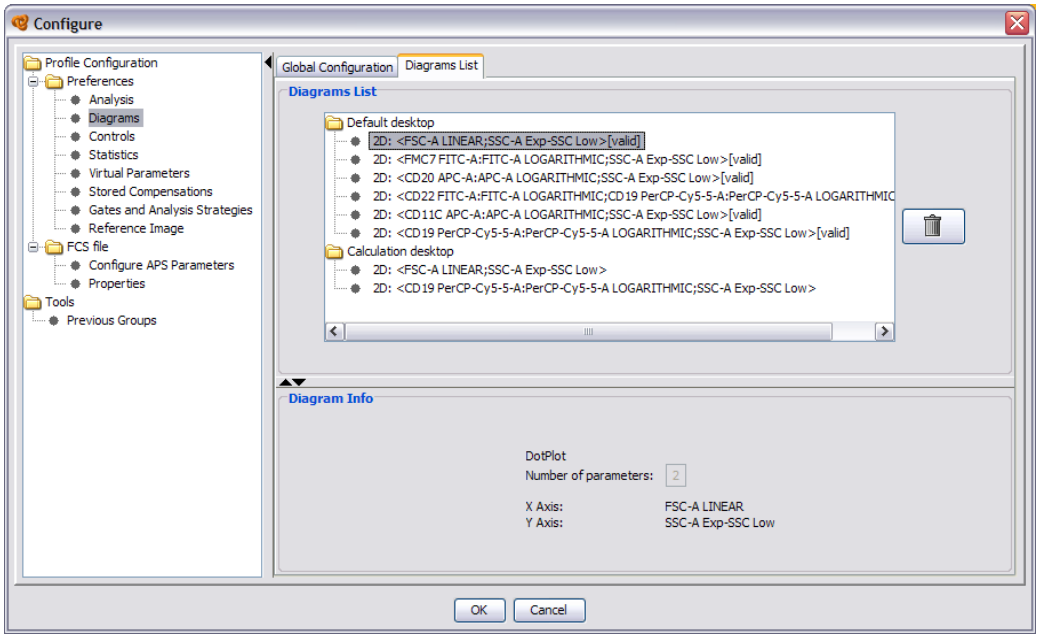
-Smooth Histogram Configuration: this option will show the same histograms but reducing the resolution of the data.

-Density Configuration: only when density is selected in some of the diagrams (right click/visualization options/density) is possible to see the changes in this part. Default

density is with dots in a colors scale, but if the user wants to show density lines as well, it is possible to do it clicking on this option of contour lines. Also the Density increment can be configured in this box.

-Global Dots Percentage: in this option you can reduce both, the number or percentage of events to display in all graphs.

A second tab in the Diagrams Configure shows the list of diagrams to show in this profile



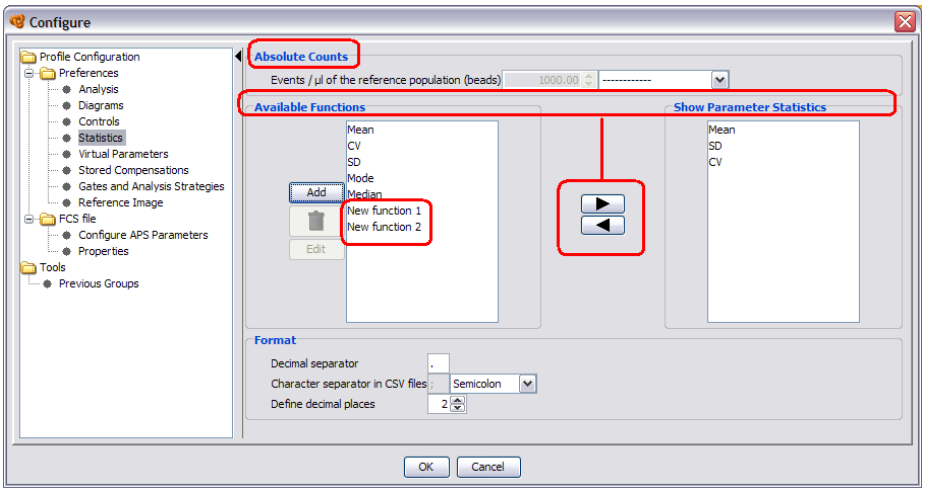
In case you have used the Calculate Data option, the software will keep in the profile the diagrams used for the calculate data wizard. Then, if you use different diagrams to select the population to calculate, which are different from the diagrams you use in your manual analysis, the software will keep both lists of diagrams and will use them.

CONTROLS

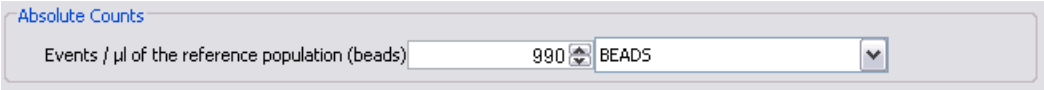
This window corresponds to the different values and commands of the Merger Process Controls are explained in detail in chapter 6.5and the controls for Calculate Data process explained in chapter 7.4.

STATISTICS

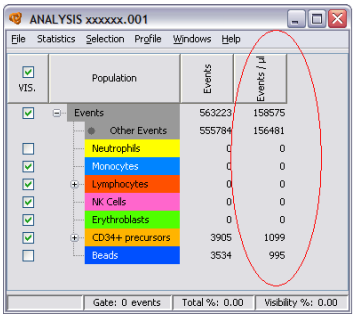
This window is used for adding as many new functions as needed.



ABSOLUTE COUNTS



If the user is working with beads for absolute counts, there is no need to calculate a formula for each sample and each population. With this tool, the user can choose which population will be taken as reference for the calculation, and with the number of events per microlitre, the software will automatically show a new column in the Analysis Window with the events/microlitre data.

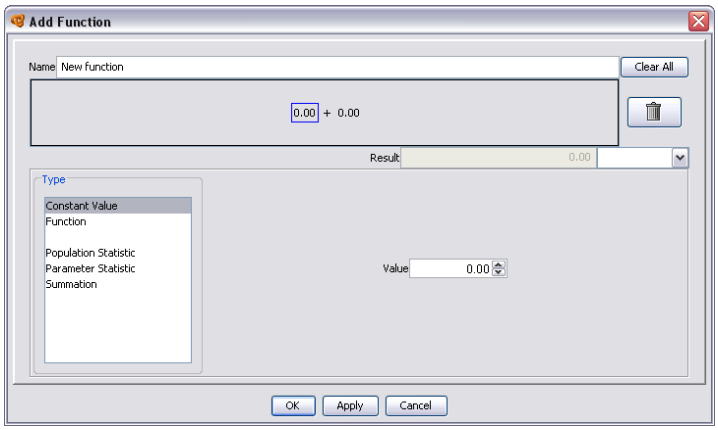


AVAILABLE FUNCTIONS

It is possible to show the *Mean*, *Standard Deviation*, *Median*, *Mode* or even a *New Function* which can be defined by the user. All the statistics available can be shown, or not, in the population tree and in the report.

The user can choose the functions of interest to show, just moving to the right with the button ► from **Available Functions** chart to **Show Parameter Statistics** chart.

In the Available functions chart, the user can Add a New Function. Then, in the box displayed it is possible to choose the name, the variables and the mathematic calculation between them.



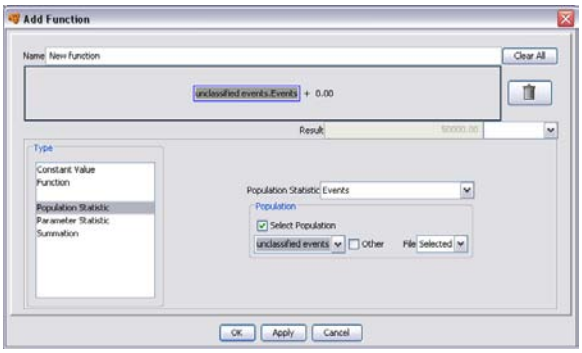
Clicking in the first part of the formula, different ***Type*** of data will be displayed so the user can choose the appropriate one.

The data may be:

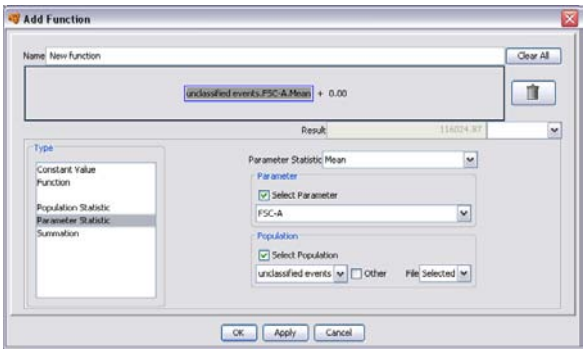
Constant value**:** The user will choose a ***Value***.

Function**:** To get a more complex function, the user can click on this choice and it will appear as a new function in the first part of the formula. This option can be repeated as many times as needed. For each component of the sub function the user will choose the type of data.

Population statistic**:** When the data consist of one of the parameters shown in the Population Tree regarding events, percentage... The user has to choose the data, the population involved and the files considered for that calculation (in case of merged files).



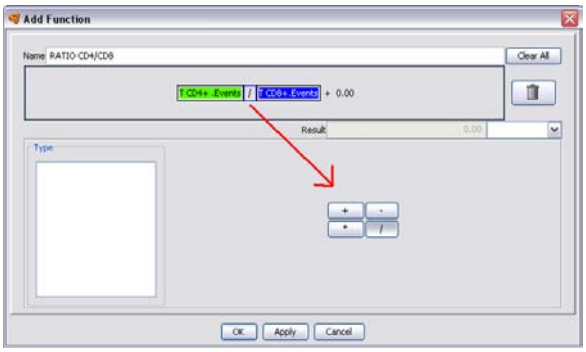
Parameter Statistic*:** In this part the user can choose the statistic value of the ***Parameter Data (Mean, SD, CV, …) for a ***Selected Parameter*** and for a ***Selected Population***.



Summation

This option is useful when the user needs to manually calculate the variables and needs the summation of a column.

Whenever the two parts of the formula have been chosen, the user can choose which type of calculation has to be performed. Then, clicking on the "+" signal, the software will display the different mathematical operations available. Then, click OK.



FORMAT

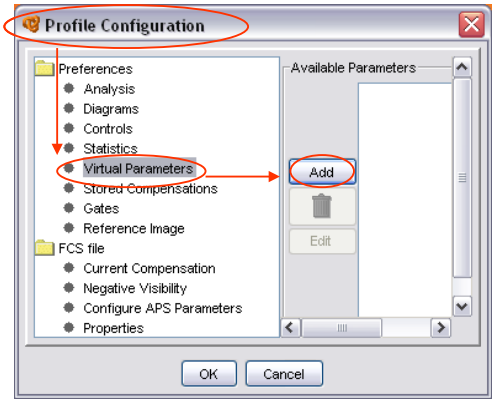
-***Decimal separator:*** For the values showed in the statistics, report and txt exportations.

-***Character separator in CSV files*:** The user may choose the appropriate value separator.

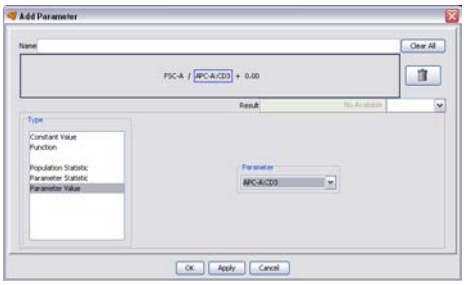
-***Define decimal places:*** It is possible to define it for the values showed in the statistics.

VIRTUAL PARAMETERS

This version of the software allows creating new parameter as a combination of others. In this sense, the way to create them is similar to create new statistics, with the difference that the new created parameter can be chosen in the diagrams to be represented.



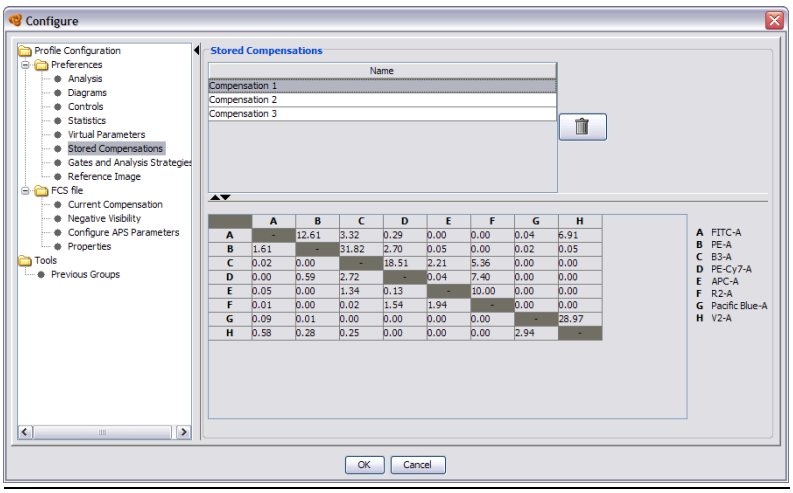
If you click on Add Parameter, the following window appears and you can give a name to the new parameter and choose which calculation you want to represent (e.g.: dividing a parameter by a constant number, rest one parameter from the other…).



Once the new parameter is created, it will appear in the list of parameters to represent in the diagrams and will contain all the statistics as usual**.**

STORED COMPENSATIONS

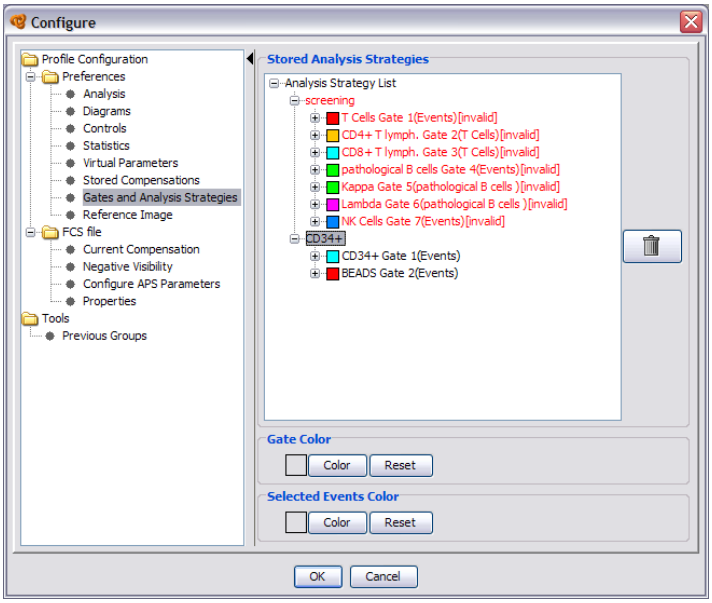
In this part of the profile, the user may have stored different compensation with different values. (See also Current Compensation Option this same section, to learn how to store them). The user can change the current compensation for one of the compensations already stored. When clicking OK, then the selected compensation will apply to the file, but whenever you open a new file, it will be always opened with its internal compensation.



GATES AND ANALYSIS STRATEGIES

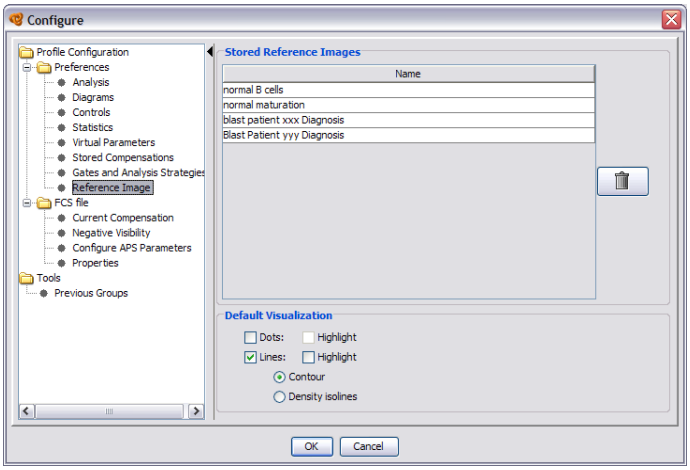
This window, as explained in chapter 4.10 shows the different Strategies saved and gives the option of checking or delete them.

In the lower part of the box, it possible to change the color of the events and the contour of the regions.



In this window it is also possible to change the color of the contour of the selection region and the selected events before an assignation.

REFERENCE IMAGE

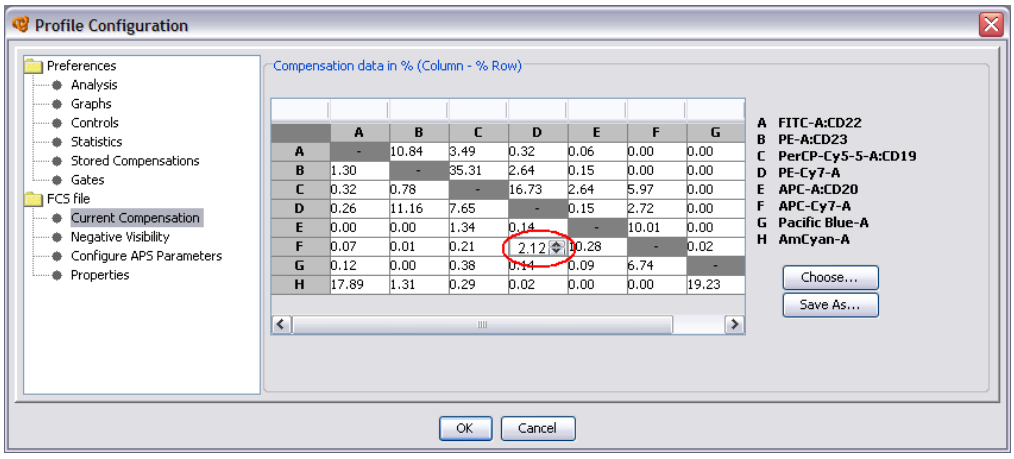


As shown in 4.9 it is possible to store a group of events as a *Reference Image* in the graphs. All the groups of events we Save as…will appear listed in the profile. Through this window we can delete them or change the appearance of the image.

CURRENT COMPENSATION

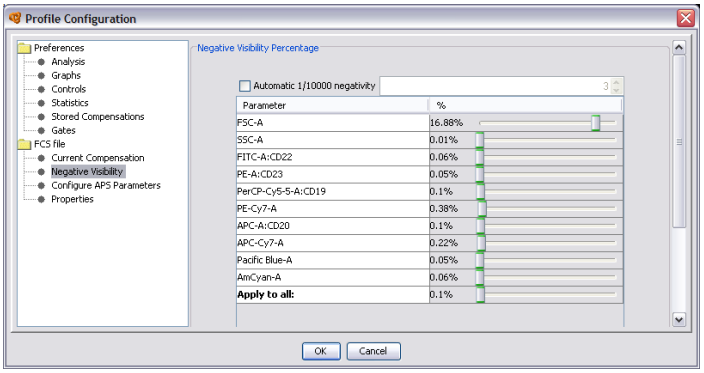
This option shows the information of the compensation values which is stored in the data file. These values can be changed manually and be Saved as…

It is also possible to Choose a stored compensation and change the values to modify it.

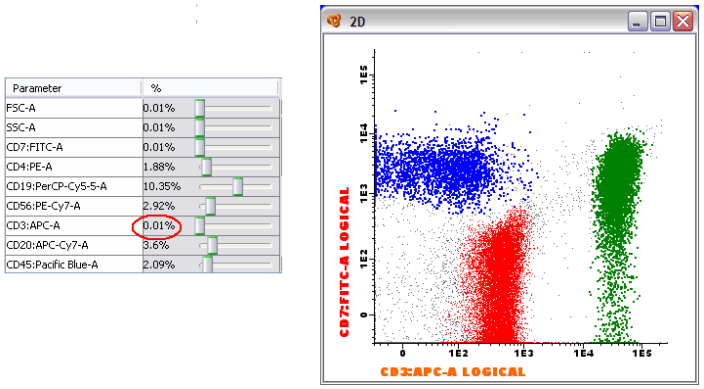


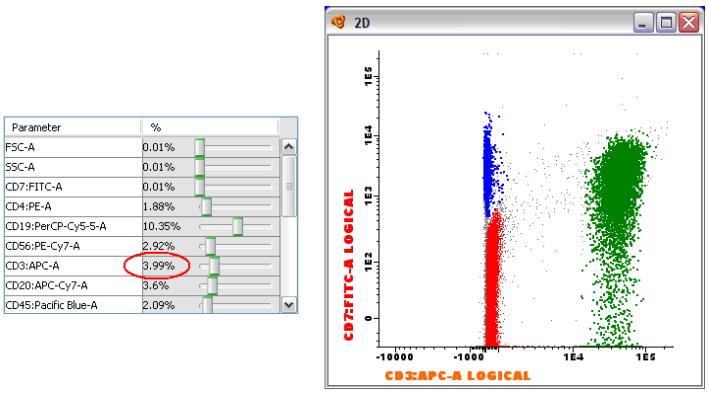
NEGATIVE VISIBILITY

These values represent the percentage for negative data range in the LOGICAL scale which is shown in the graph. It corresponds to the part of the negative axes in relation to the positive axes.



The percentages shown for each parameter can be moved to improve the visualization. Depending on this percentage, the negative part of the scale of the parameter will be less represented. The maximum value is 20%.

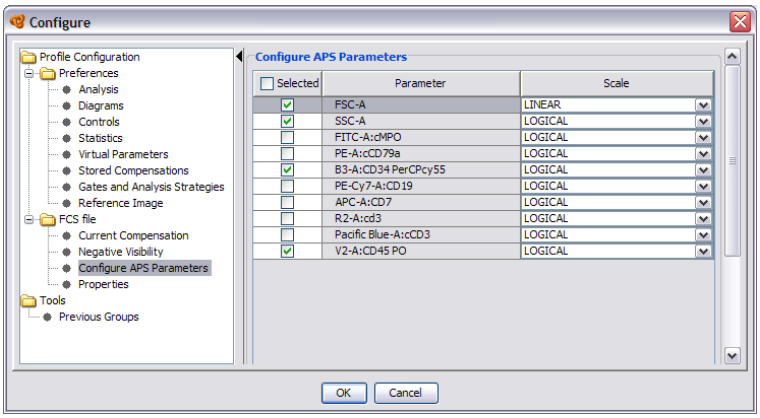




CONFIGURE APS PARAMETERS

This option allows eliminating from the profile one or more parameters for the APS calculation. Remember that this action will apply globally while it is always possible to do it locally from each APS graph in the right click menu /Diagram Configuration /APS parameter Configuration.

The software will take into account only the parameters selected.

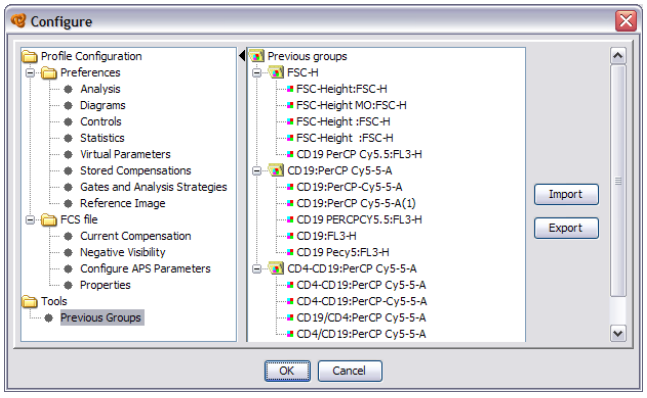


PROPERTIES

This window corresponds to the one previously described on section 4.13.1.8 about the characteristic of the parameters on a given data file.

TOOLS: PREVIOUS GROUPS

This part shows all the Group actions done (6.4) and stored in the computer. As can be seen in the figure it is possible to import or export dictionaries of labels from different users or computers.



5. PRINTED REPORTS

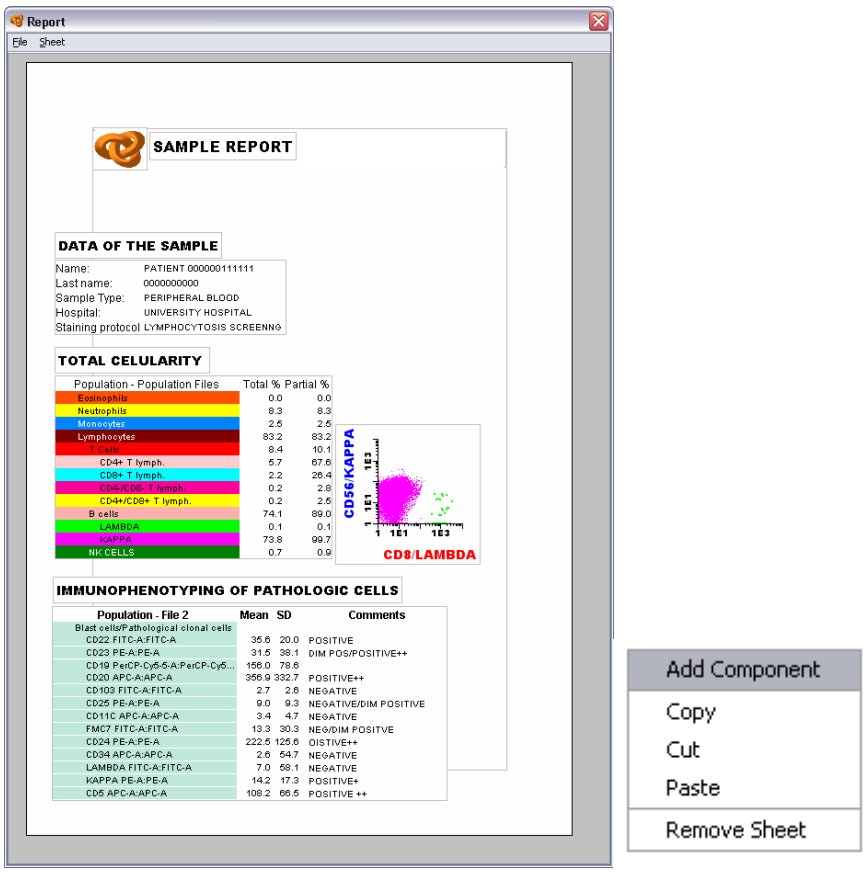
The printed report is a key element of the analysis and is part of the profile. Once the user completes the analysis, it is possible to define a customized summary of all the information which can be printed and stored. The analysis report will be updated each time a new file is loaded.

Infinicyt™ gives the user the possibility of editing his/her own model of printed report in a very easy way consisting of handling different elements such as graphics, statistics charts, text, pictures,…and all the different elements the user wants to include.

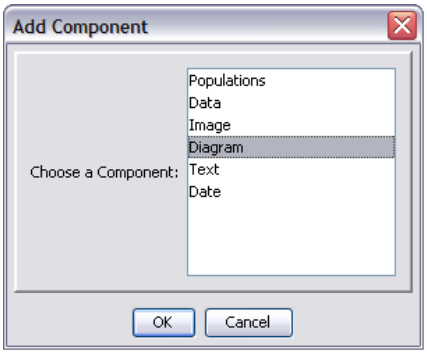
5.1. How to create a Report

Go to **File** menu and click **Print.** A report page opens (see figure) with different components with standard information about the analyzed populations and the data included in the file.

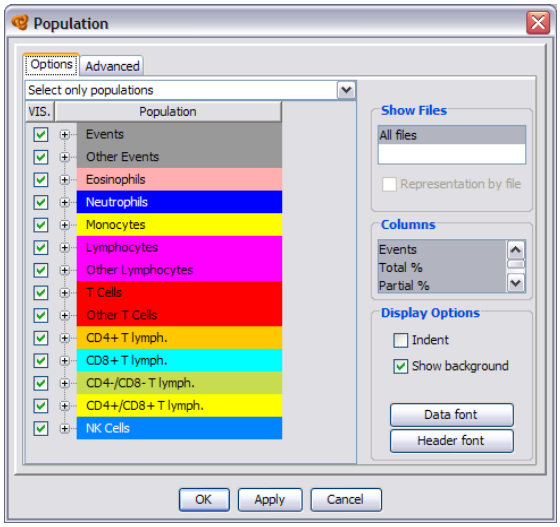
Each component can be deleted, just right-clicking on the component and choosing "**Remove Component**"



You can complete this standard report, deleting and adding some elements, by right-clicking in the middle of the report. A menu will appear with the different elements you can include (population trees, data, image, diagrams or text). All of them can be moved and resized in the report page.

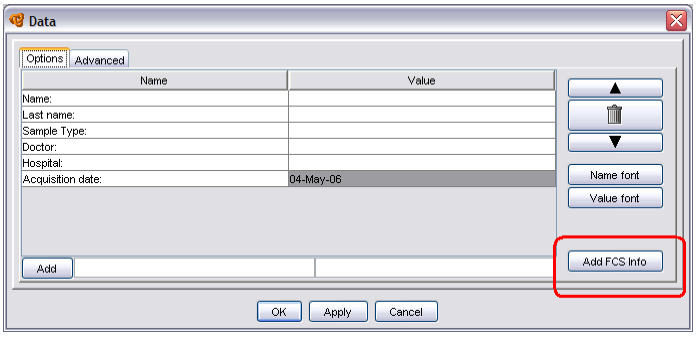


Each of these elements will also have a menu, for example, if you choose **Population**, you will obtain a list of your populations. If you double-click on it, you will obtain a complete menu where you can choose the populations and parameters to be shown on the report.

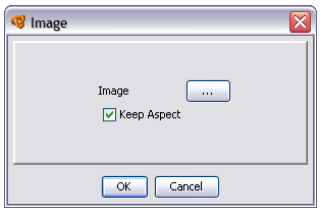


If you choose **Data**, a box with some sample data will appear as a default. Clicking twice on it you can configure this element and choose the data of interest.

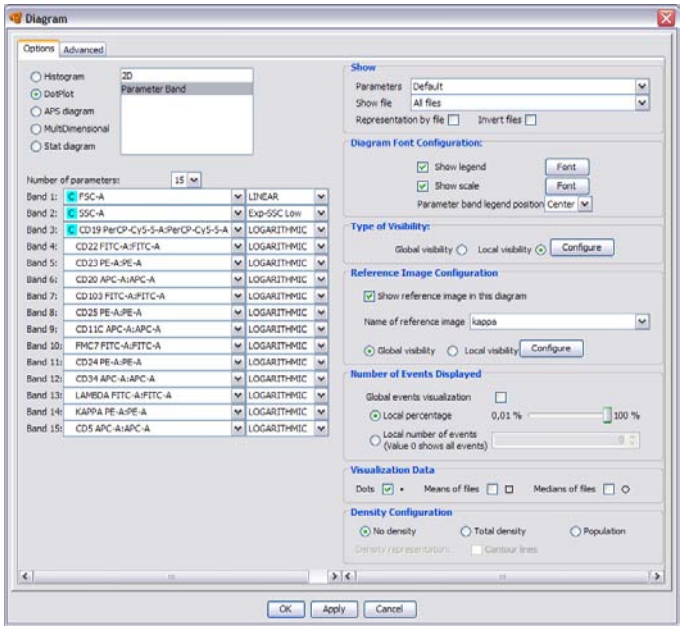
All the fields will have to be filled any time you want to do a report of a new sample. If you want this data to be automatically updated, then select all the fields from the FCS info, and will be directly read from the data file.



If you choose **Image** you will be able to insert a picture or a logo stored on your hard disk.



You can also choose a **Diagram**, and a dialogue box will open to choose the graphic of your interest (see figure). As you can see the diagram can be configured on parameters, visibility, density,...etc.



Apart from choosing the type of diagram, you can configure the diagram in the report with the following options:

Show

-Parameters/ Population: in case of Parameter Band dotPlot or Parameter Band Histogram, you can directly choose to show as many bands as *Populations and parameters with comments*, *Populations with events*, or *default* to configure one by one. In this case of parameter band graphs, you can ask for the representation *by file* (the band will be at the same time divided in files) and even invert the order that the parameters will appear in the graph.

-Show File: in case of merged files, it gives you the choice of show *All files*, *population files*, or *each file* independently.

Diagram Font Configuration

To choose legend and scale to be shown, and the font size.

Reference Image Configuration

From version 1.4, it is possible to show a Reference Image on the diagrams of the report. In this part the user can choose which image to show and even, with the visibility, choose the order of populations included in the Reference Image to "send to front" the one of interest. It is also possible to configure it if we prefer the Reference Image with lines, dots, hightlighted…

Number of events displayed

Same as in the right click of the diagram, here you can configure the number of events to show.

Visualization Data

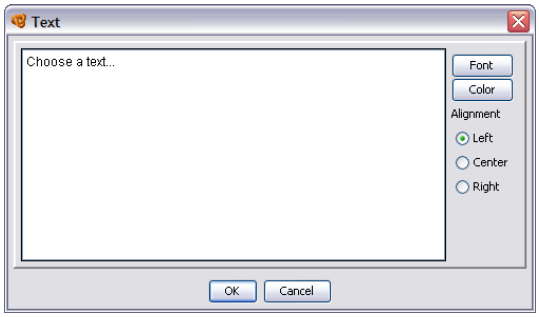
It is possible to choose Means, Median, Dots or a combination of them.

Density configuration

Here are included the same options as in the diagrams menu.

All the diagrams here configured will be kept in the profile, and will be shown updated; any time you run a sample with the same parameters and populations analyzed.

If you choose a **Text**, a dialogue box will ask you about the text you want to write, color, font and alignment.



Finally, choosing **Date**, a box with the current date will appear.

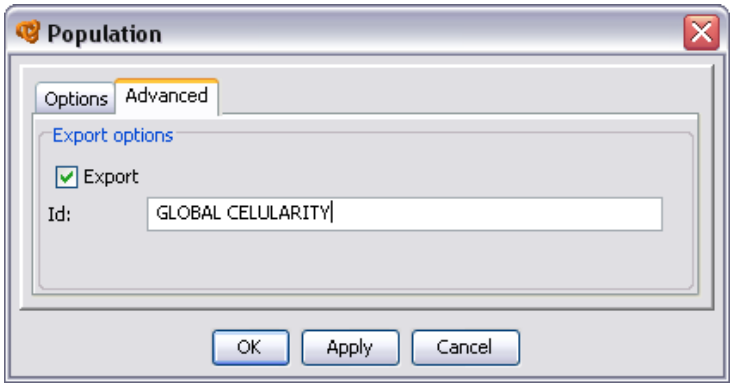
All these components can be moved and resized in the report, and will update with new data as soon as the user change the data file in the analysis.

While the report is open, no gates or operations can be done in the analysis.

5.2. Export Report to XML

For each of the components of the report you can choose whether to export or not to XML files. This can be done by clicking twice on the component to configure, and in the advanced tab you can select or not the export option.

You can also write a name for this component so any system will identify the component.



The XML files generated in this way, can be read by customized external programs that may allow import data to LIS.

6. FILE MERGE

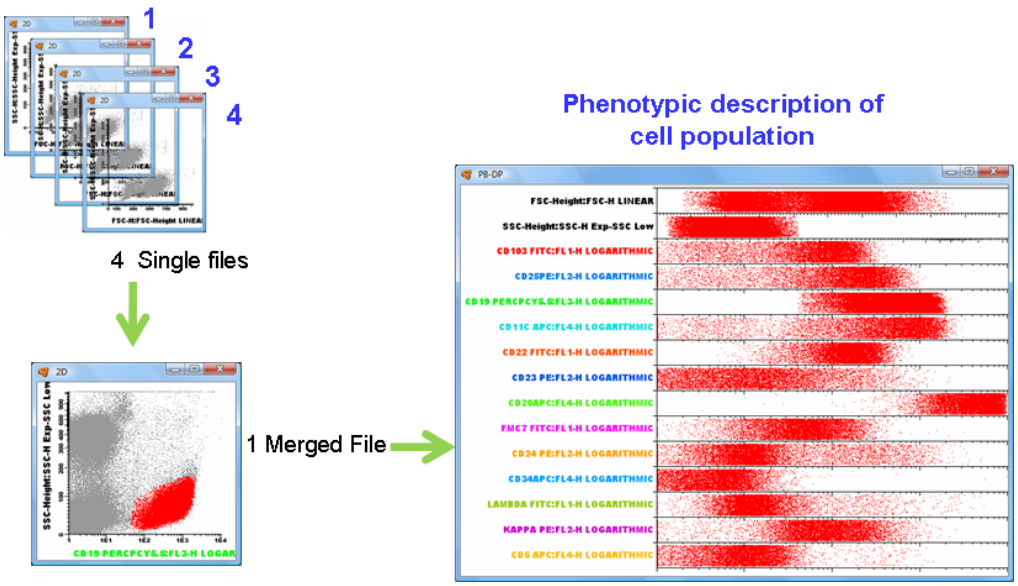
6.1. Introduction and merge conditions

With this function, the user can select several FCS files and merge its information in a single file.

The file resulting from the merger will contain all the events of all the files selected for the merge process. For example, if you merge 4 files containing 50.000 events each, the result will be a file containing 200.000 events.

All the parameters measured will be merged into a single data file with “n” parameters, but each parameter is saved separately.

As you can see in the following figure, after the parameter-based merging process the user may study the expression of all the markers of the panel in the same data file.



6.2. Merge conditions for global analysis

Infinicyt™ will merge all the data files required, but if the user wants to perform a global analysis on all the tubes at once, be aware of the following:

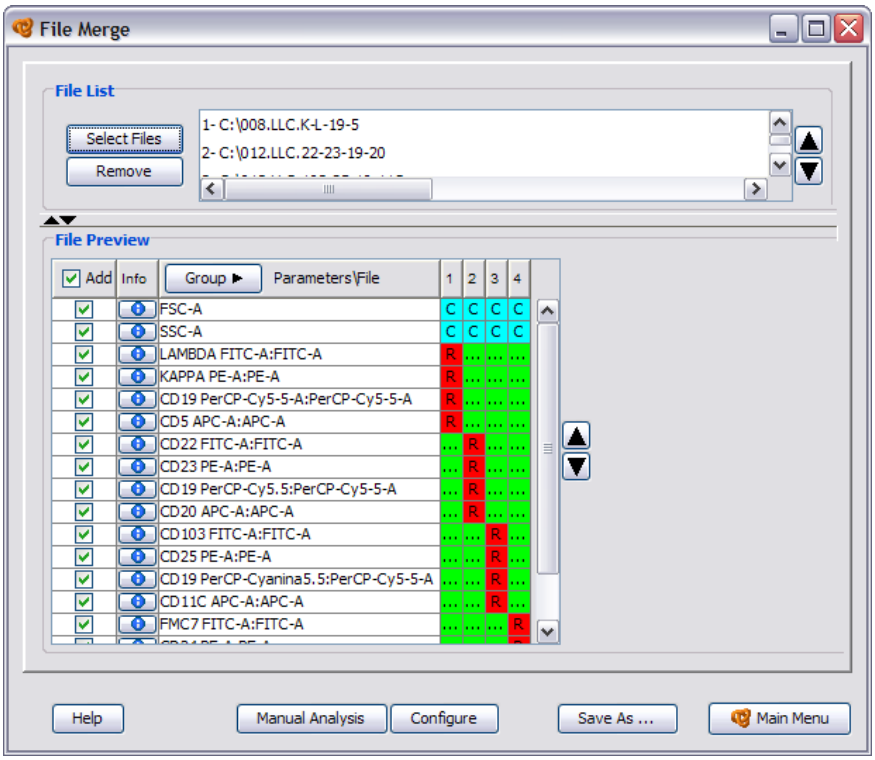
-Acquisition settings : The parameters and files must be similar regarding acquisition settings (PnE, PnR and Data Type). If not, the software will try to adjust the scales and will show you a warning.

-The appropriate marker panel design is essential in order to obtain satisfactory results with merger mode. The design of the necessary antibodies panel must be done taking into account that in each tube of the panel should appear:

- A series of common parameters that allow to unequivocally identifying the population of interest for our particular study. The more common parameters we use, the more precise the identification of the population of interest will be, e.g. to identify the population of B lymphocytes from a peripheral blood sample, FSC, SSC and CD19, at least, could be used as common parameters.
- It is necessary to include in each tube all the markers that will allow us to identify the population of interest (common parameters), e.g. if our population of interest was the helper/inducer T cells, all the tubes in the panel must have at least CD3 and CD4 markers for their correct identification.
- The common markers used in the panel must be marked with the same fluorochrome.
- In fluorescences not occupied by common parameters, the rest of the antibodies to be used in our study will be included.

Infinicyt™ includes an internal control system that verifies if the aforementioned conditions apply (See chapter 6.5).

6.3. Merge Process



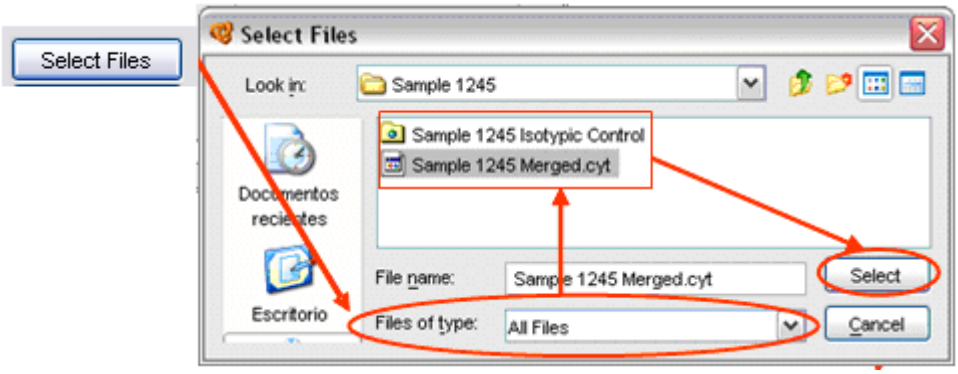
- **Select files**: this command opens the directory where the user can select the files to be merged. Click **Shift** to select consecutive files in the folder or **Ctrl** to select alternative files. Once the selection is done click **Select**.

- **Remove**: with this button you can select a file to remove from the list of files to be merged. If no file is selected and you click this button all of them will be erased.

  You can select and move the files on this list with the▲ and ▼ cursors, in order to obtain the best sequence to represent the tubes in the graphics. The first one will be the closest to the axis in the graph representations.

  Once you select the data files, the matrix (parameters/file) appears in the lower part of the dialogue box. In this matrix, the user can **Add** or not each parameter, in order to avoid parameters that are not interesting because there is no marker on it. With this, we will optimize the memory of the new data file reducing the number of parameters to be merged.

  You can merge a **FCS** file with an already analyzed file **.cyt.** In this case of merging files, in the step you are selecting them, you will have to choose “all Files” in the option “Files of Type” in the save window, so you will have both **.cyt** and **.fcs** files available to merge.

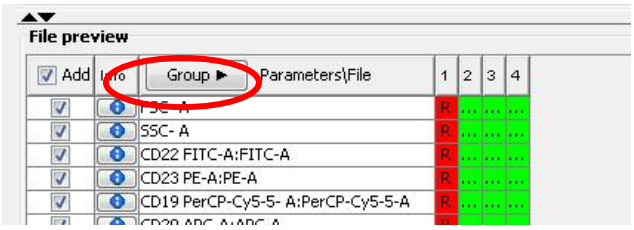


- **Group**: with this action you will be able to correct typing mistakes of the labels by writing a new name for them and which parameters should all have exactly the same label. Detailed information of this tool can be found on next chapter 6.4.

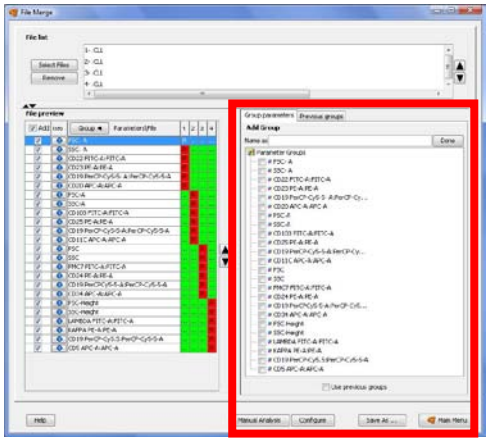
- **Manual Analysis**: with this button, you can go directly to Manual analysis without saving to disk the new file resulting of this process. The new merged file will be open and ready to analyze but without saving. This can be done to avoid memory space, if you prefer to check and analyze your file before saving.
- **Configure:** File merge options allow manipulating the controls that will warn you in case your common parameters are not similar enough to perform a merge process. See chapter 6.4 for more information on this.
- **Save as…** when the selection of the data files is done and the parameters to be merged are the correct ones, the user can save this information. The software will ask you for a new name and directory for the new FCS file.
- **Help**: document with the User´s Manual chapter of this Module will be displayed.
- To go back to the main screen of the program, click the **Main Menu** command.

6.4. Group tool (Dictionary of labels)

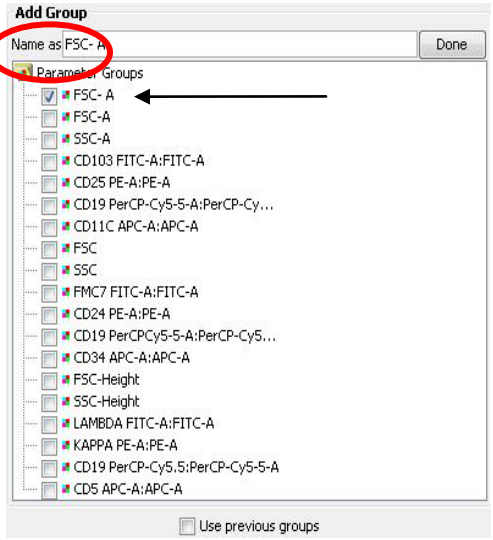
Infinicyt™ 1.4 includes a new tool to solve the problem of merging parameters with different labels, which improves the Goup tool of version1.3. When there is a label discrepancy on parameters that should be common, just click **Group**.



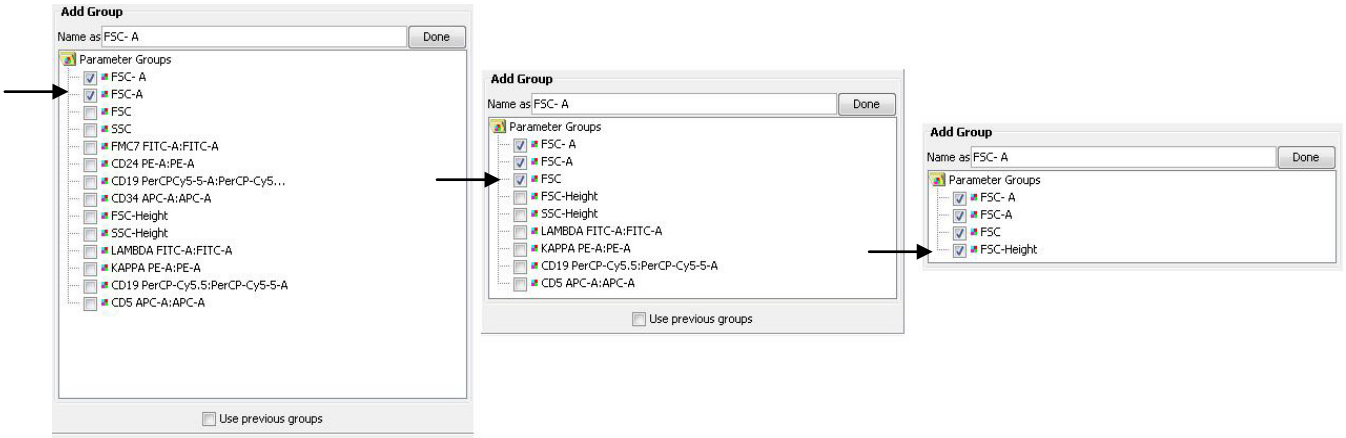
It appears a dialog box in which the user may click on the labels that should be grouped.



The first label selected will give the name to the grouped parameter, but it can be changed in the box **Name as**.

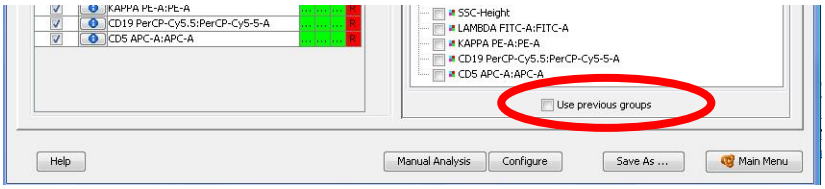


As soon as a first label is selected, some parameters disappear from the list. These are the ones that belong to the same file, therefore can not be grouped with the same name.

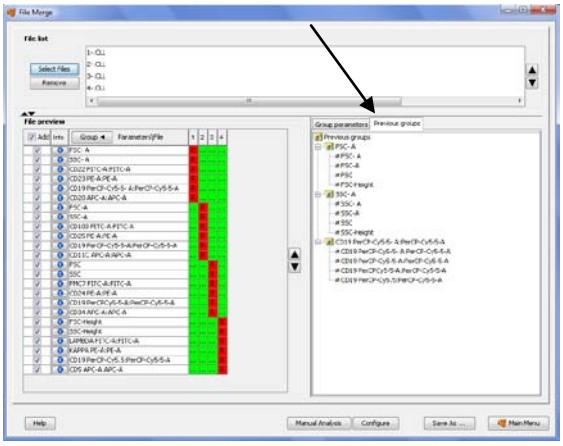


Once all the desired parameters are selected, click Done. Automatically the matrix will change to show the grouped parameters as Common.

At the same time, the software will store these actions in **Previous Groups** and will be possible to remember them later. It means that at the same time the user creates groups of labels, the software creates a dictionary. The second time we load these files to make a merge, the software will make the groups automatically, if we click on **Use previous groups.**

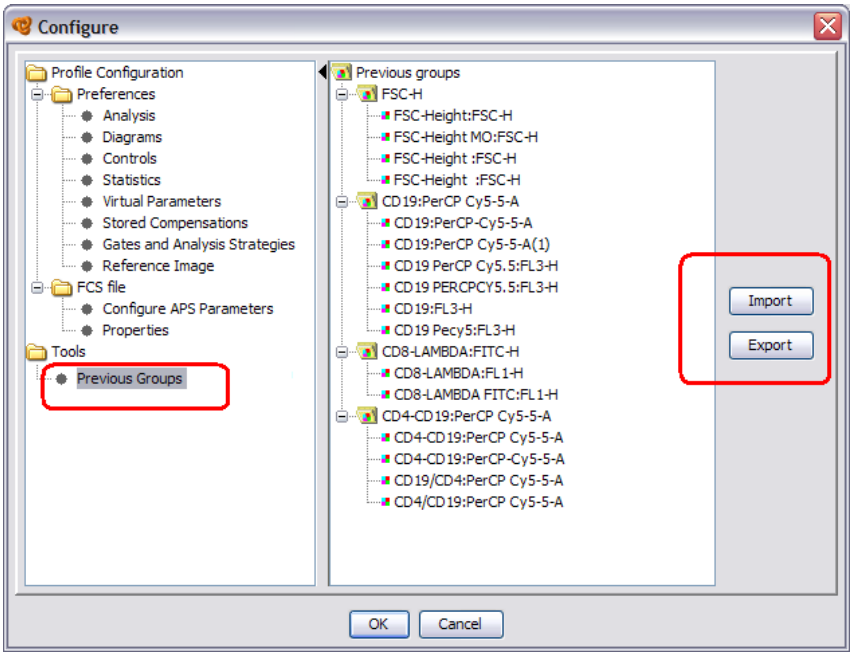


The previous groups already done can always be checked in this tab of the window.



The previous groups information can also be checked in the Manual Analysis Window Menu Bar, option **Profile /Configure /Tools /Previous Groups**. In this case the previous groups stored will be saved in the application Infinciyt™, instead of be stored on the profiles. It means that it will be always available to use independently of the Profile it is open each time. The Dictionary contains Drag and Drop tools to move the labels and the groups.

The group's configuration can be exported as a file .dic (dictionary) and imported from this window.



6.5. Merger process Controls

Infinicyt™ includes a process of **Coherence control** to determine if the user is performing the merge process with the optimal files, analyzing in different aspects the similarity of expression in the common parameters.

The control contains different threshold values, so the user can modify them in the corresponding option. Nevertheless, the users are strongly advised **not** to change the threshold values predefined in the program, when working with real data, unless they understand perfectly these processes. Failure to comply with this warning may produce unreliable results.

There are two major types of coherence controls:

-Global coherence control (Merge Control): the software analyzes the similarity of the total events from each file between all files merged regarding the common parameters.

-Populations coherence control (Population Control): whenever the user analyzes a merged file, and tries to define a group of events from different tubes as a unique population, the software analyzes the similarity between these populations from each file.

In this sense, the *Merge Control* allows to compare the main populations represented in the file, to detect, for example, that a common MoAb has not been added, while the *Populations control* allows comparing populations less represented which can go unnoticed in the global coherence control.

In both controls, there are some threshold values to define the strictness of the software controls to show a warning of no coherence between files or populations. The threshold values, explained in the next chapter, can be obtained in the Manual Analysis Window Menu Bar, option **Profile/Configure/Preferences/Controls** menu.

6.5.1. Merge Control

WARNING OF NO COHERENCE BETWEEN FILES

This validation process evaluates the global image differences between the respective distributions of events of the different tubes, in the n-dimensional space defined by the n common parameters. In the current version, this image difference is represented by a number from 0 to 100, as follows:

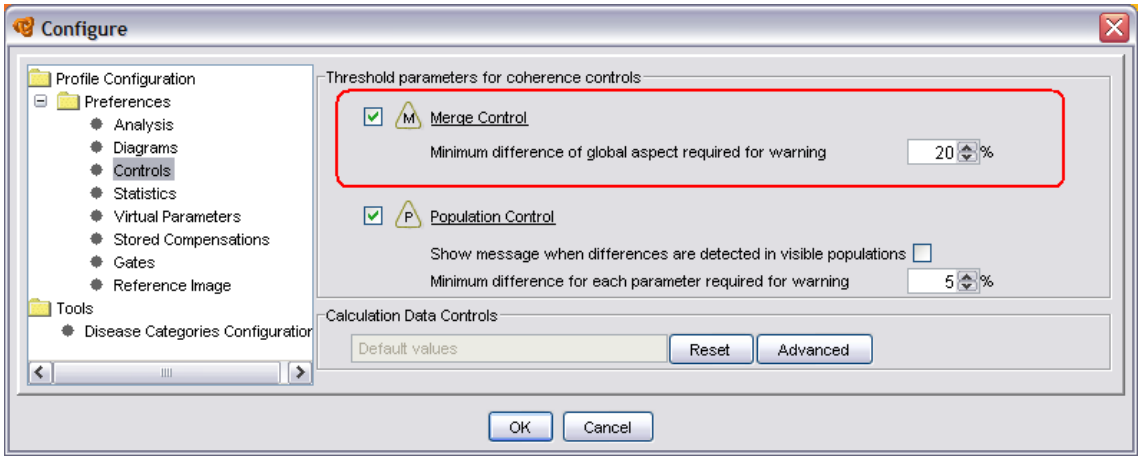
- value 0: no significant difference, the distributions are identical.

- value 100: the distributions are completely different.

The values between these limits represent different degrees of similarity. Although, in general, values close to 0 will indicate very similar distributions, and those close to 100 very different ones.

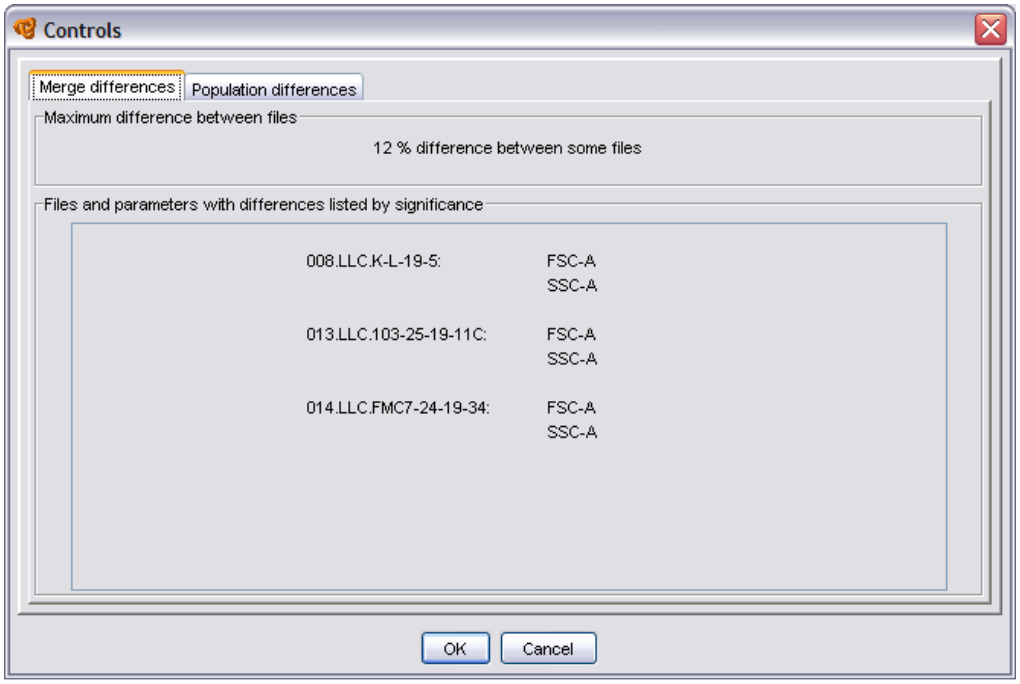
To activate this control, check the **Merge Control** option.

The parameter indicated as "**Minimum difference of global aspect required for warning**" contains a threshold value configurable by the user, which determines the minimum degree of difference in the scale from 0 to 100 explained above, between any couple of files, over which the program will issue a warning indicating a significant lack of coincidence between the distributions of the events of the different files. Current predefined value: 20%.



When the program detects significant differences between the distributions of the events of the different files in the common parameters space, as determined by the aforementioned threshold values, it shows a warning message.

The warning will indicate the % of difference and will also list, by significance, the parameters and files which make the differences.

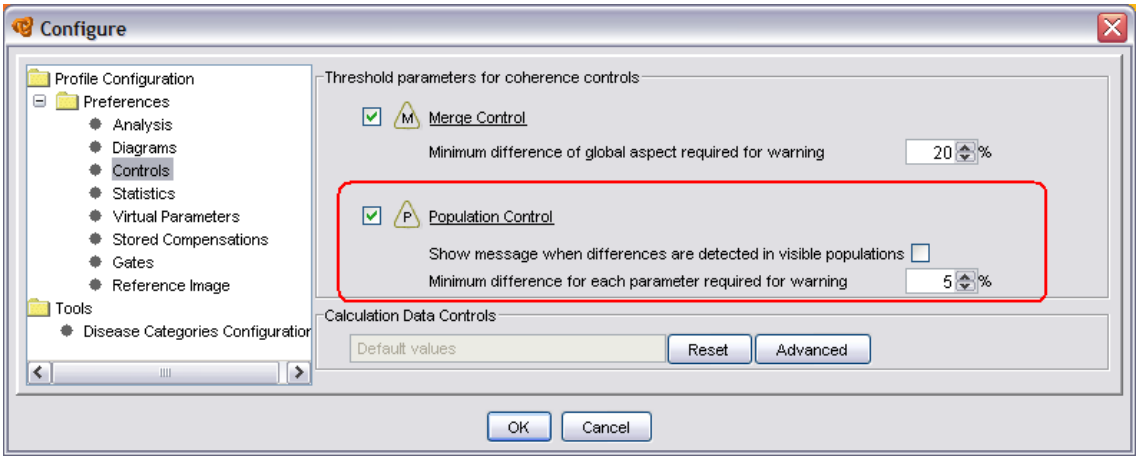


In the analysis window of a merged file we will find a CONTROL button for MERGE process If it is shown on red, and click on it, a window with the Population differences will be listed. If there are no differences, it will remain green.

6.5.2. Population control

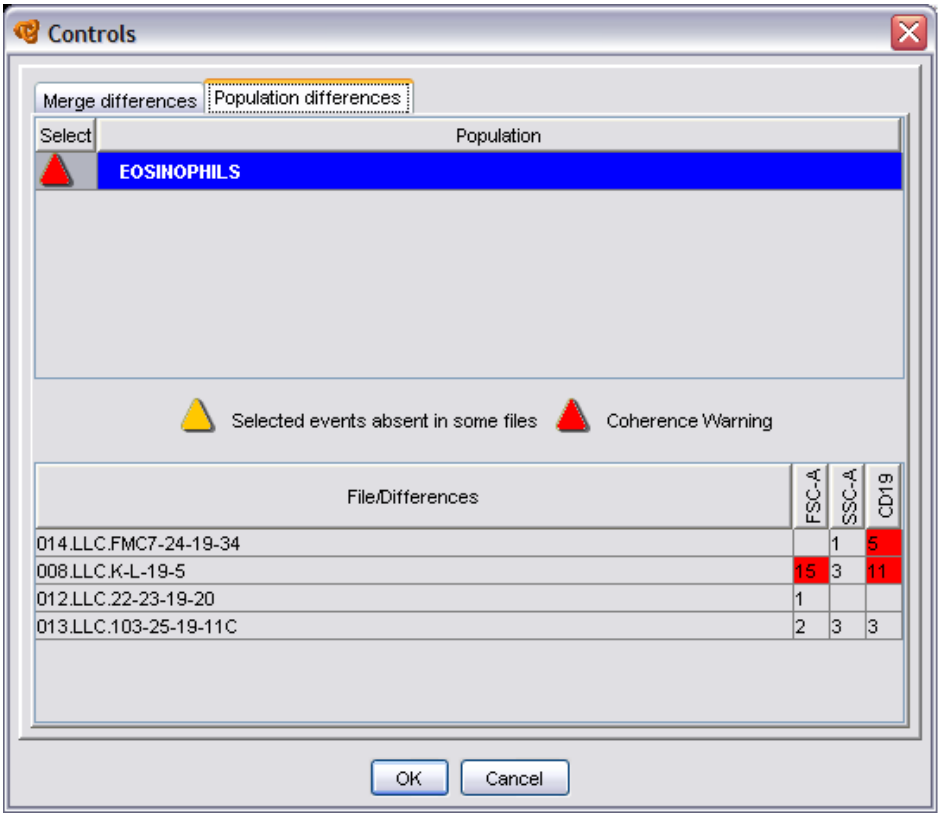
This second control evaluates the similarity of cell clusters from different files that we consider within the same population. This control operates once the files are merged and the user starts to analyze the populations.

To activate this control, check the **Population Control** option in this part.



This validation process operates on each defined population in a merged data file; the process evaluates, for each common parameter of the population, the possible image differences between the one-dimensional distributions of this parameter in the different files. This image difference will result in a warning message if there are at least two files for which the difference between the mean values of this parameter plus the difference between the standard deviations, relative to the total parameter range, exceeds the value set by the user for the threshold value **"Minimum difference for each parameter required for warning"**, which has a currently predefined value of 5%.

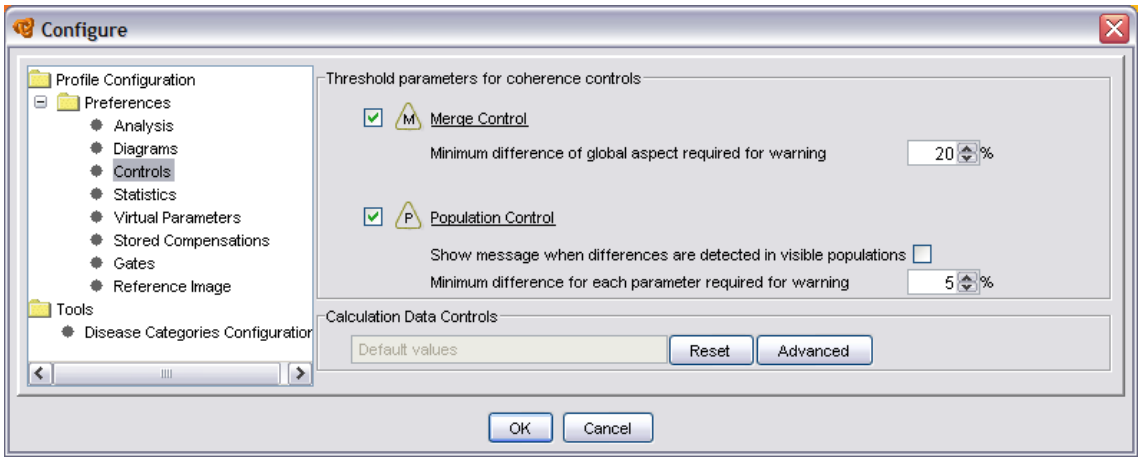
In the analysis window of a merged file we will find a CONTROL button for POPULATION . If we click on it, a window with the Population differences will be listed, in case there are.



This button will appear red (▲ Coherence warning) whenever any population of the *Population tree* is over the fixed threshold of coherence. In the right part of this box we will see the files and parameters with incoherence.

This button will appear yellow (▲ Selected events absent in some files) whenever the selection performed in common parameters does not contain events from all the tubes, as could be expected when working on a dot plot with common parameters.

If we click in the Manual Analysis Window Menu Bar, option **Profile/Configure/Preferences/Controls** window we will see the values taken as threshold (same window as *Configure* in the *Merge* process), and we will be able to correct these values.



As previously described, the commands for fixing the warning thresholds shown above, can be obtained in 2 different ways when the user is working with a merged file:

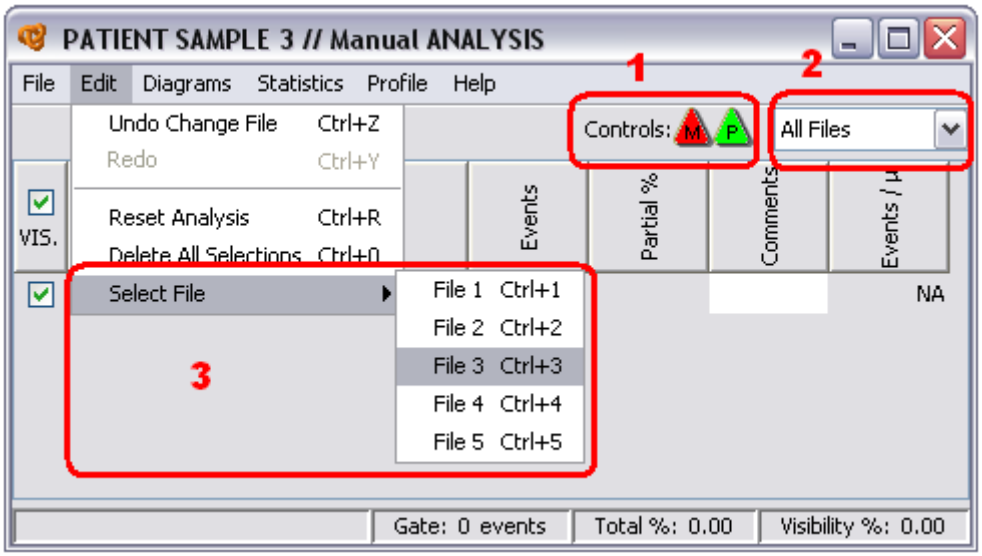
1-**Profile/Configure/Preferences/Controls** from the Analysis Window menu. The commands

of the analysis window change to red when an incoherence is detected.

2-From the File Merge window, in the option **Configure.**

6.6. Analysis of the merged files

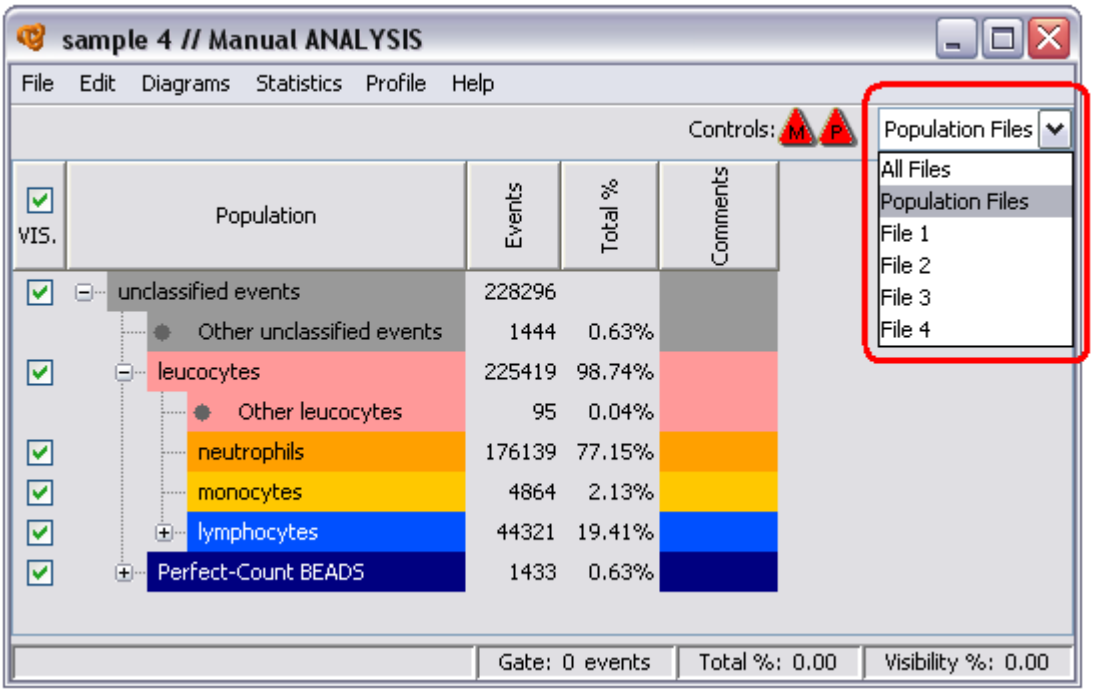
Once you go to the Manual Analysis option, note that if the user is working with a merged file, the *Manual Analysis Window* includes some functions useful in this analysis.



-**1**: **Control buttons:** this buttons will appear on red color if the software finds important differences related to the coherence controls among the files (M=Merged Control, P= Population Control). See controls information in next chapter 6.5.

-**2**: **All Files.** Whenever the user is working with a merged file, the statistics and percentages (*Total* and *Partial*) can be given as data from the total merged file, data from each original file or data from the files containing the chosen population.

For this purpose, a pop-up menu is present in the right-upper side of the Analysis Window, where the user may select the information regarding all files, only from files in which population is present or only from each of the tubes.



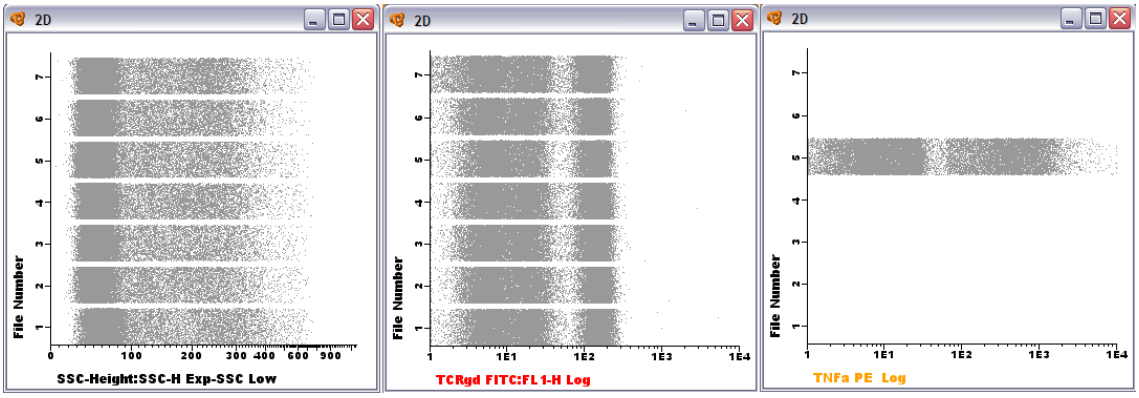
If you want that the files appear with the file name instead of the file number, you can change it in **Profile/Configure/Preferences/Analysis/Show in analyzer.**

The expression NA (non applicable) will appear in the statistics in case there is no way of giving a logic calculation of a percentage of a population included in different files.

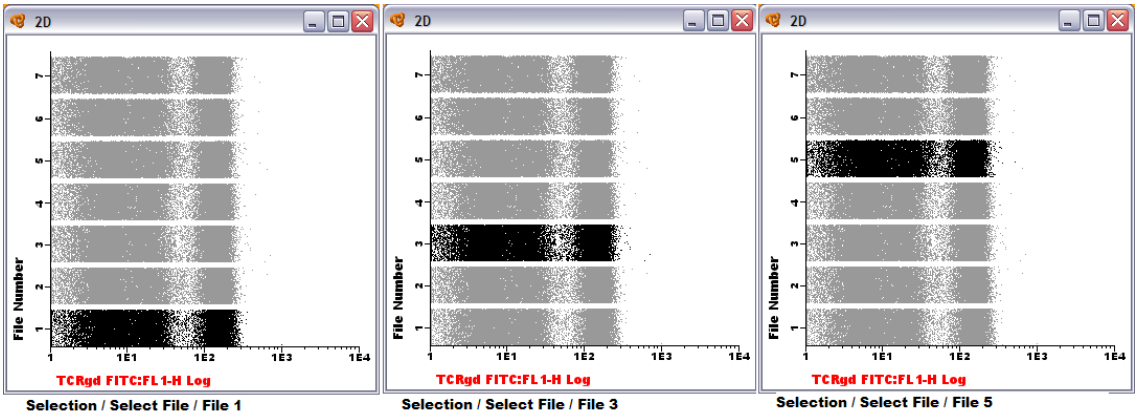
-3 Possibility of **select gating per file** (**Select File**) when the user is interested only in gating all the events from one of the original tubes. See more information in chapter 4.13.2.4. The new merged files contain the new FILE NUMBER parameter, which will allow us to separate the events corresponding to the original files using a FILE NUMBER representation.

In this sense, if a parameter is common to all files, it will be represented with all the bands, and a parameter which has only been measured in a file will be represented only in that band.

In the next figure, the parameters SSC-Height and TCRgd-FITC have been measured in the 7 files and are considered by the software as common parameters, while the parameter TNFα-PE has only been measured in file number 5 and only those events are represented.



In this representation, the user can also perform a quick gate on all events of each tube with the option **Selection** / **Select File** / …or **Ctrl+1**, **Ctrl+2**,.. (see chapter 5.1.2.4).


2D
File Number
TCRgd FITC:FL1-H Log
Selection / Select File / File 1
Selection / Select File / File 3
Selection / Select File / File 5

7. CALCULATE DATA

This option is only available in the Advanced Version of the software.

7.1. Description

The user can calculate several characteristics of cells in which a parameter has not been directly measured, based on the knowledge of those characteristics in phenotypically similar cells which have already been measured in a different aliquot.

Then, once the same cells have been measured in different aliquots, the software will find for each cell, the equivalent one on the other files and will apply the values for the other markers not directly measured on them.

This process is valid to study phenotypically identical cells in which is not possible to measure all the characteristics in a same aliquot due to parameters limitations.

In order to merge and calculate the parameters, the original files must have as many as necessary common parameters that allow the identification of the population in which we want to calculate different parameters. The necessary number of common parameters, as well as the appropriate marker panel design is fundamental in order to obtain satisfactory results with the calculate data.

	FILE No								
PARAMETER	1	2	3	4	5	6	7	8	9
FSC-HEIGHT	C	C	C	C	C	C	C	C	C
SSC-HIGHT	C	C	C	C	C	C	C	C	C
CD11b-FITC	R								
CD13-PE	R								
CD45-PerCP	C	C	C	C	C	C	C	C	C
CD34-APC	C	C	C	C	C	C	C	C	C
CD2-FITC		R							
CD56-PE		R							
HLADR-FITC			R	R					
CD117-PE			R						
CD123-PE				R					
CD15-FITC					R				
CD16-PE					R				
CD22-FITC						R			
CD25-PE						R			
CD65-FITC							R		
7.1-PE							R		
CD61-FITC								R	
CD33-PE								R	
CD71-FITC									R
Glycophorin-PE									R

CALCULATE DATA

	FILE No								
PARAMETER	1	2	3	4	5	6	7	8	9
FSC-HEIGHT	C	C	C	C	C	C	C	C	C
SSC-HIGHT	C	C	C	C	C	C	C	C	C
CD11b-FITC	R	D	D	D	D	D	D	D	D
CD13-PE	R	D	D	D	D	D	D	D	D
CD45-PerCP	C	C	C	C	C	C	C	C	C
CD34-APC	C	C	C	C	C	C	C	C	C
CD2-FITC	D	R	D	D	D	D	D	D	D
CD56-PE	D	R	D	D	D	D	D	D	D
HLADR-FITC	D	D	R	R	D	D	D	D	D
CD117-PE	D	D	R	D	D	D	D	D	D
CD123-PE	D	D	D	R	D	D	D	D	D
CD15-FITC	D	D	D	D	R	D	D	D	D
CD16-PE	D	D	D	D	R	D	D	D	D
CD22-FITC	D	D	D	D	D	R	D	D	D
CD25-PE	D	D	D	D	D	R	D	D	D
CD65-FITC	D	D	D	D	D	D	R	D	D
7.1-PE	D	D	D	D	D	D	R	D	D
CD61-FITC	D	D	D	D	D	D	D	R	D
CD33-PE	D	D	D	D	D	D	D	R	D
CD71-FITC	D	D	D	D	D	D	D	D	R
Glycophorin-PE	D	D	D	D	D	D	D	D	R

1 SAMPLE: 9 TUBES : 9 FILES

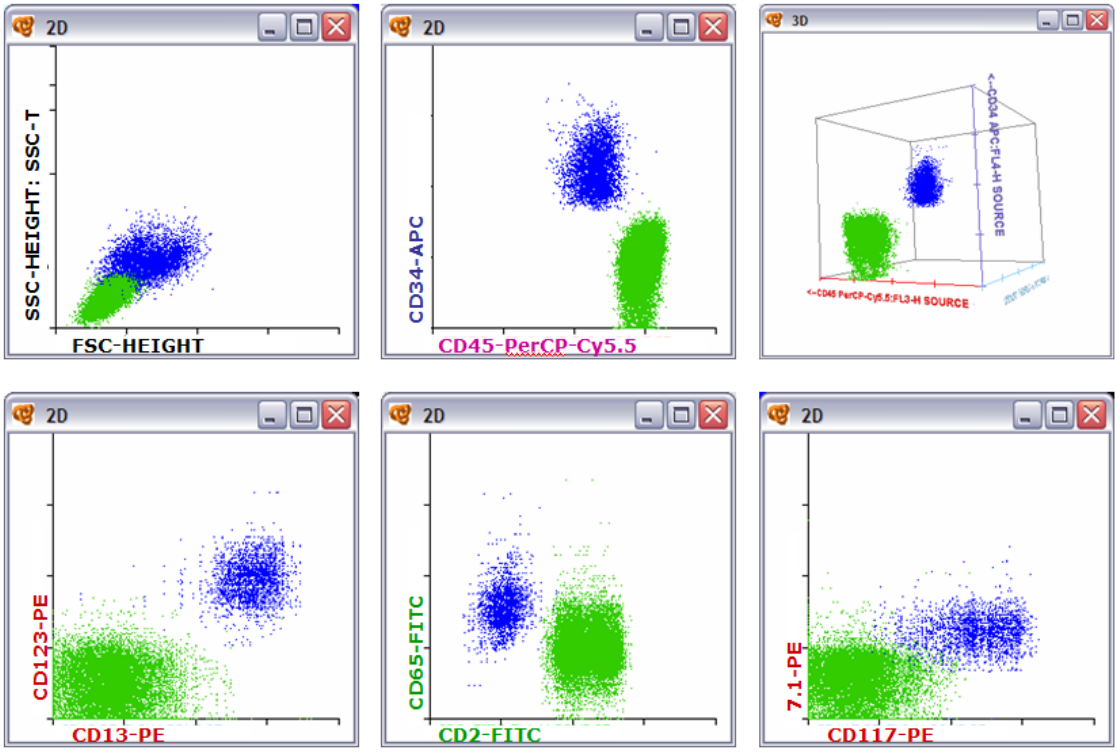
1 SAMPLE: 21 PARAMETERS: 1 FILE

The tables show an example of 9 tubes that make up a panel, with the analyzed parameters of each tube.

Characteristics that have been measured repeatedly in all tubes (common parameters) are marked with a "C" and will be used by the program to base its calculation of similar cells. Parameters actually measured in a tube (real parameters) are shown with an "R" and the new calculated data of these parameters with an "D".

Infinicyt™ multidimensionally reconstructs the new data files containing both the real and calculated data for those parameters not directly measured.

The overall number of parameters in the new file can be as large as the total number of parameters measured in the whole group of tubes. This allows a more powerful identification and characterization of the different populations, with data from all the markers of the panel within each cell in each tube (see following figure).



In order for the calculation algorithm to work correctly, the FCS files that you want to calculate have to meet the following requirements:

Regarding the panel design, the common parameters of the population to be calculated have to exhibit different characteristics, i.e. markers expressed differently in the same cell populations should be in the same tube.

The acquisition settings have to be the same for each acquired tube to be merged. The software is able to harmonize the FSC/SSC characteristics in surface and

cytoplasmic staining, to search for the same selected cells in the different files. Other common parameters apart from FSC and SSC can also be harmonized.

Infinicyt™ includes an internal Calculate process control system that verifies if all the previously mentioned conditions apply, although it is always possible to carry out a control of the Calculate Data comparing the calculated results with a real file.

WARNING: Validation of Calculated data mode has to be done for each user with its own panels.

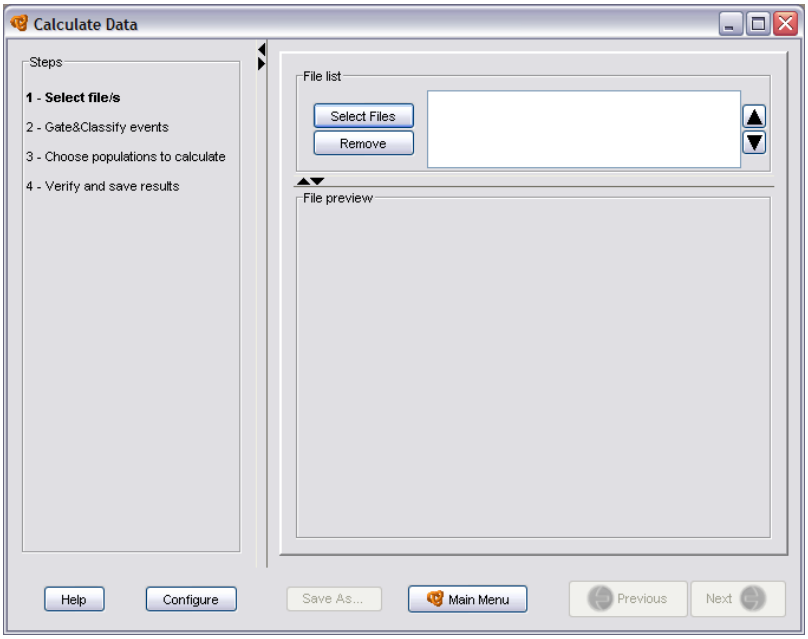
7.2. Limitations

This module has been validated under conditions where identical cells can be unequivocally selected with the Common markers included in the panel. As an example, clonal pathological cells from B-lymphoproliferative disorder fit the conditions of being identical (clonal) therefore if they are appropriately selected with common markers, the information from the different tubes can be calculated in the others.

7.3. How to use the Calculate Data wizard

Calculate Data has to be used following different steps.

Whenever you click on the Calculate Data in the Main Menu, you will obtain a wizard with the different steps you will have to follow:

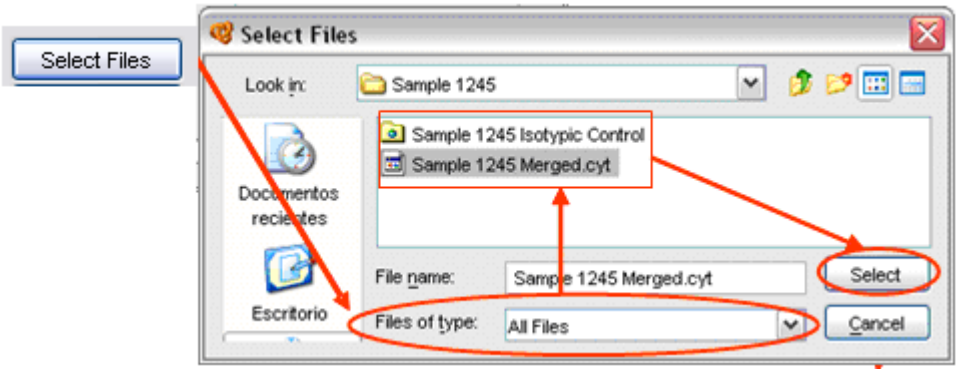


STEP 1: SELECT FILE/S

In this first step, the user has to select the files in which the data has to be calculated. You can select either independent files or you can select an already merged file. In both cases, the software will consider them as a merged file and the window will show a matrix as it is shown in the merge screen.

Select files: this command opens the directory where the user can select the files to be merged and calculated. Click **Shift** to select consecutive files in the folder or **Ctrl** to select alternative files. Once the selection is done click **Select**.

If you want to calculate and already merged and analyzed file, you will have to choose "all Files" in the option "Files of Type" in the save window, so you will have .cyt files available to calculate.

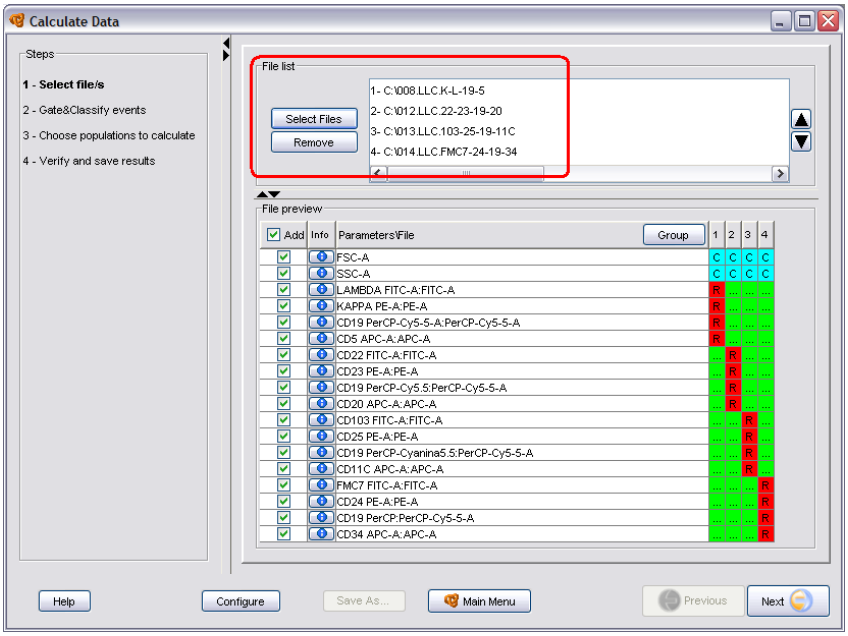


Remove: with this button you can select a file to remove from the list of files to be calculated. If no file is selected and you click this button all of them will be erased.

You can select and move the files on this list with the ▲ and ▼ cursors, in order to obtain the best sequence to represent the tubes in the graphics. The first one will be the closest to the axis in the graph representations.

Once you select the data files, the matrix (parameters/file) appears in the lower part of the dialogue box. In this matrix, the user can **Add** or not each parameter, in order to avoid parameters that are not interesting because there is no marker on it. With this, we will optimize the memory of the new data file reducing the number of parameters to be merged.

For more information about Merge process, go to chapter 4.2.



If some of the labels of the common parameters are different and they are not classified as common, then, you can click on "**Group**" to change the labels. See chapter 6.4.

Once you are sure that these are the files and parameters you want to calculate, click **Next** on the wizard to follow with the next step: Gate and classify events.

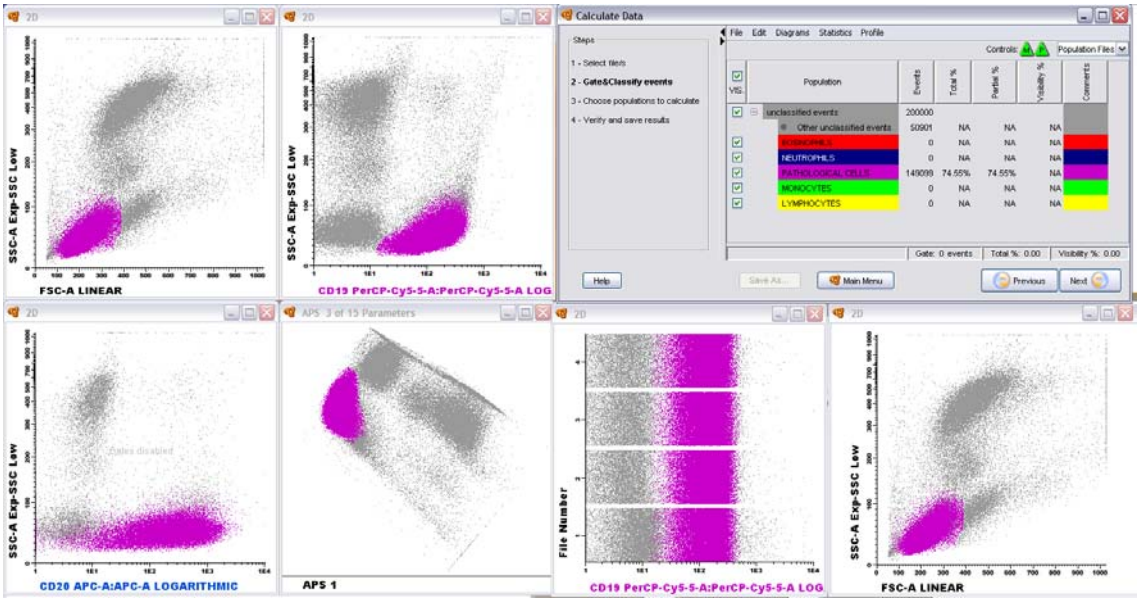
STEP 2:GATE AND CLASSIFY EVENTS

In this second step, the software shows a desktop (set of Diagrams) similar to manual analysis but with certain limitations. The goal of this step is to select manually which is the population to obtain information based only on common parameters.

You can gate and classify as many populations as you want to calculate, but only in diagrams with common parameters represented. You will find the comment "**gates disable**" in some diagrams if you are showing no common parameters, because in these diagrams you won't be able to select events in all the files at the same time.

Therefore, it is recommended to create some new diagrams only with common parameters. If the panel is correctly design, the common markers will be enough to clearly identify a homogeneous population that will be later calculated for all the rest of the parameters.

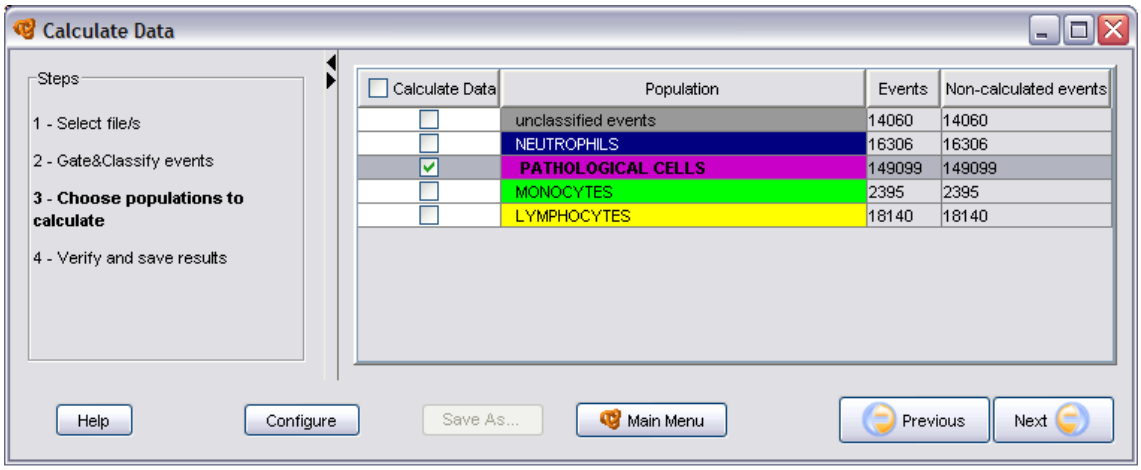
The diagrams you create will be saved as a new desktop in the profile that will be showed only when you are performing this step of the Calculate Data.



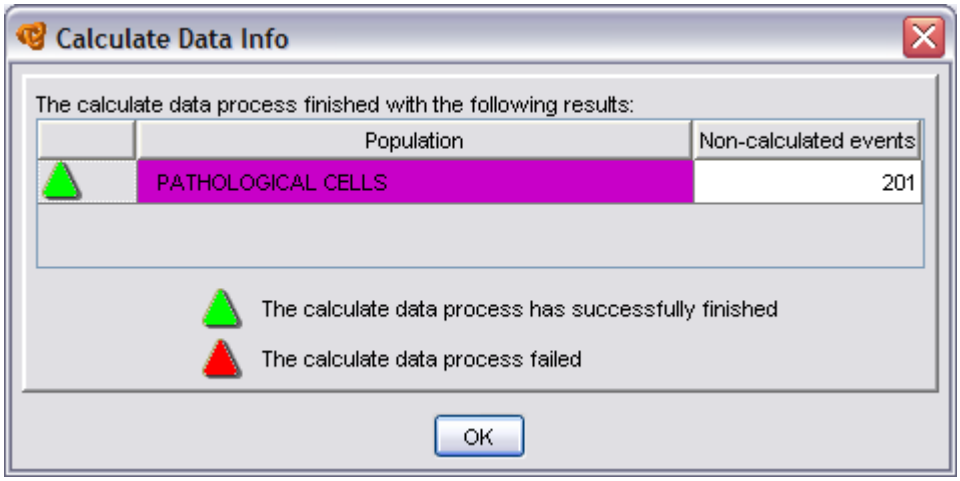
Once the population/s of interest to calculate are selected and classified in a color, click **Next** on the wizard to follow with the next step: choose populations to classify.

STEP 3: CHOOSE POPULATION TO CALCULATE

In this third step, all the populations with events classified in the previous step will be listed in this window. The user has the opportunity to choose which population to calculate is and avoid unnecessary calculation with debris and other cells. At this moment, all the events are non-calculated until you go to the next step.



Once the populations are selected, click next to start the calculation process and go to the next step. In this action is when the software performs the calculation process. It consists on finding for each of these cells the equivalent one in the other aliquots (tubes), and then applies the values of the parameters not directly measured. As soon as it ends the process, it will appear a summary with the number of events none calculated by the controls on each population. In the next step the user can see in detail those events. Controls are detailed in chapter 4.3.3.



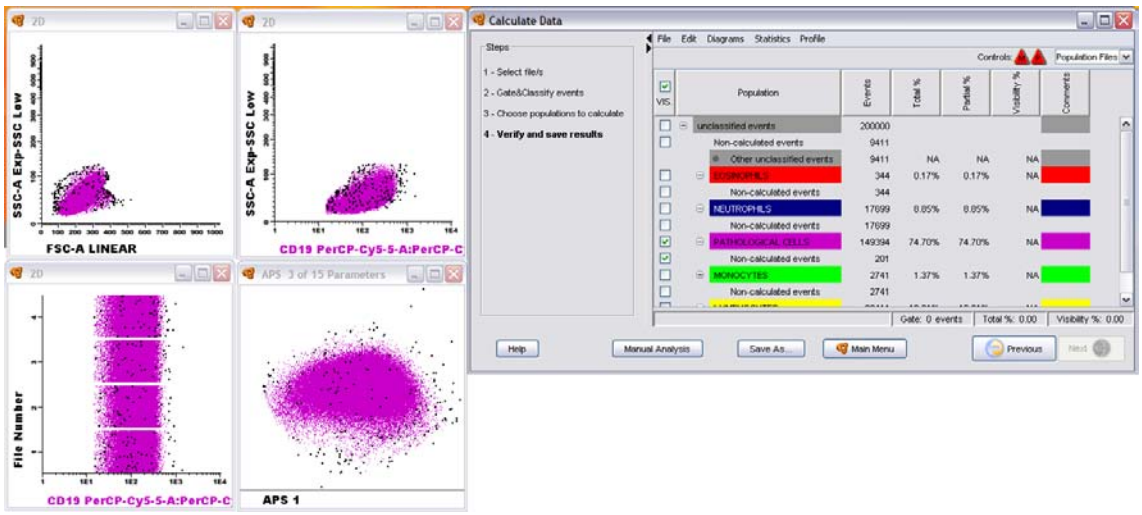
Click OK to continue the process.

STEP 4: VERIFY AND SAVE RESULTS.

In this step, the user directly goes to a pre-manual analysis (with certain limitations as described in step 2), and will be able to check the not calculated events because the will be painted in highlight within a label under the original population.

In the following figure, the violet events have been calculated and can be visualized in all the parameters. On the other hand, black events have been rejected by the controls, and the can be visualized only on the real markers for each one.

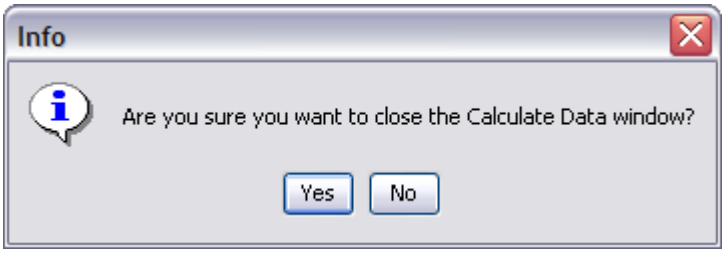
Then, it is easy to visualize only those events and paint them in a different color, as debris or as part of the population again. If you click next without painting black events in a different color, then, those events will be included as part of the original population.



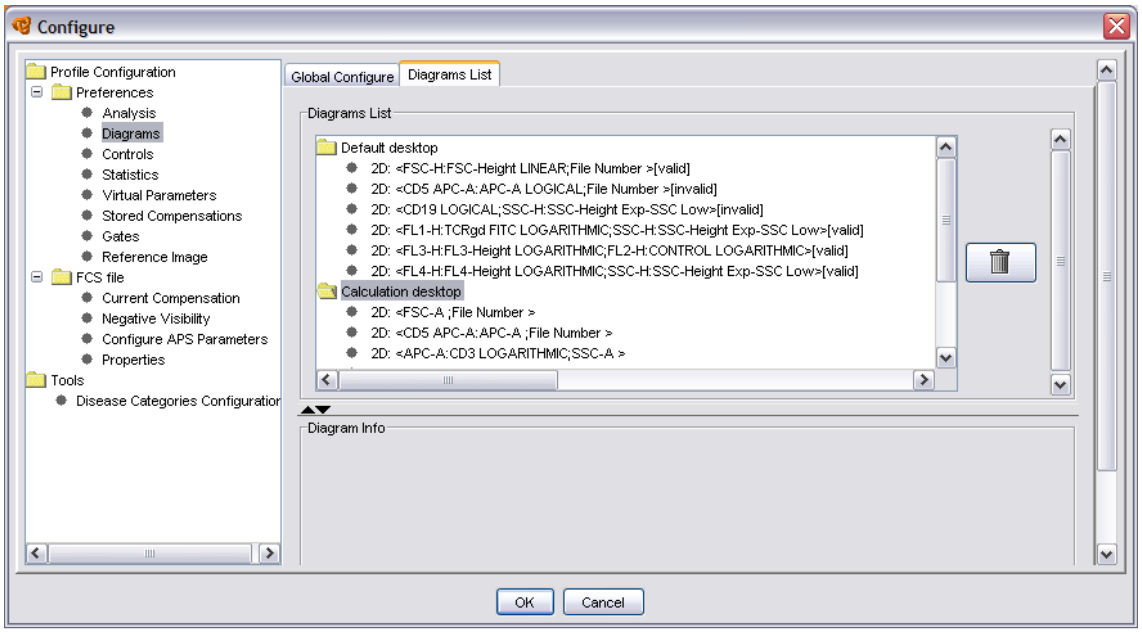
Once the non calculated events are verified, it is time to save this file as a .cyt (if we want to maintain the population analysis) or as a .fcs file if we want to save only the calculated events.

If you don’t want to save the file, you can go to the Manual Analysis to analyze this sample without saving to disk.

Both if you go to Manual Analysis, or Main Menu, you will find the following warning before closing the Calculate Data wizard.



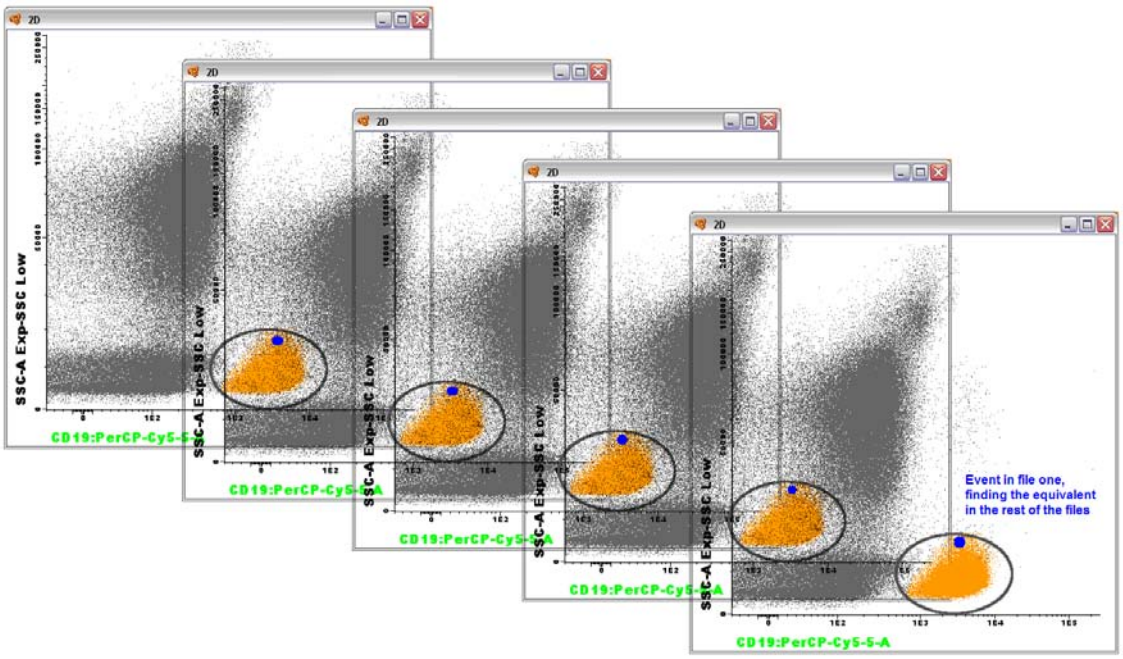
The diagrams you used for Calculate Data will be kept as part of the profile as a second list of diagrams that will always be open when you open again the Calculate Data wizard. If you go to Profile/Configure/Diagrams, you will find a second option called Diagrams list. In this box you will see that apart of the diagrams of the profile, you have saved a calculation desktop with the diagrams you need anytime you perform a Calculate Data process.



7.4. Calculate Data Controls

In the wizard you can see the bottom Configure, which can be used to set the merge and calculate controls.

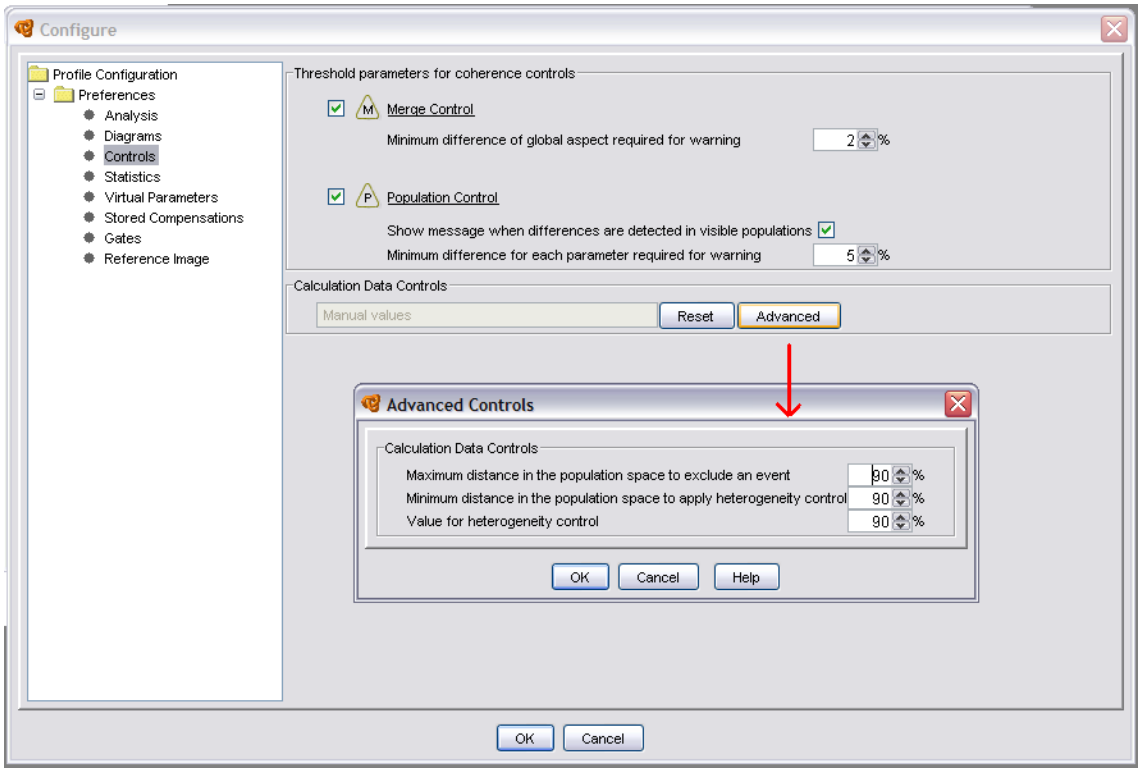
During Calculate Data process in a given population, the software will try to find for each event the equivalent event in the other files (based in the position for the common markers) and then apply the same values of the rest of the parameters. The way to find the equivalent event is by choosing the nearest neighbour in the same position.



Even more, when the common markers have some differences in the expression, the software is able to find the equivalent cell in the other file.

In this part is where the controls apply: depending how homogeneous are the populations, the area to find the nearest equivalent neighbour can be bigger or smaller.

In the Calculate Data wizard you will find the Configure button. If you click this button, you will see the controls dialogue box of the profile, with the Calculation data controls where you can manually configure the following values:



Maximum distance in the population space to exclude an event: considering 100% the maximum distance possible to find it, we can reduce this distance to exclude any event as valid for being the nearest equivalent neighbour.

Minimum distance in the population space to apply heterogeneity control: considering 100% the maximum distance, the user can reduce the area in which any event is valid as neighbour, and the events out of this region but inside the maximum distance control will need to apply heterogeneity control.

Value for heterogeneity control: for those values inside of the first value, and out of the second value, the user can establish how heterogeneous the populations are.

8. FILE EDITOR

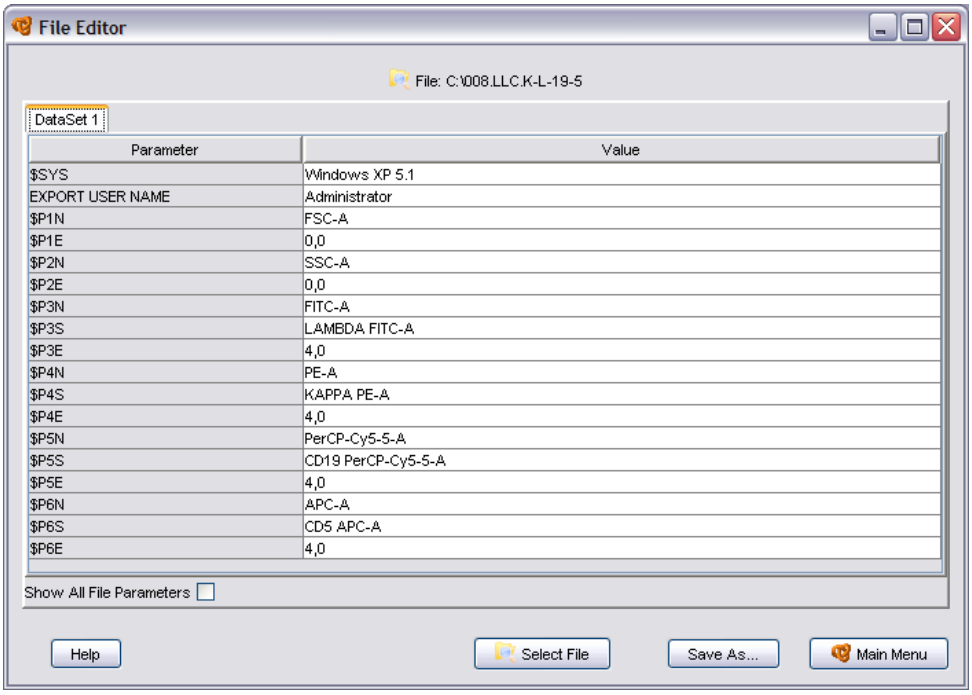
8.1. Introduction

With this module, the user may perform limited changes on FCS files, such as correcting typing errors in the parameter labels, occurred during acquisition, in case they may interfere in the merger process.

The program allows only the modification of text values of the FCS file, and only of those whose modification would not affect the coherence of the data (for example, the long names of the cytometric parameters can be modified). Please note that, to register a parameter as common to the different tubes, the software must recognize exactly the same name in the parameter description in the FCS file of each different original tube. In case there are differences, for example regarding capital letters, dots, hyphens, commas, script, etc., the software may not identify the corresponding parameters as common. Therefore, it is fundamental to check the spelling of the parameters that are going to be common.

8.2. Available commands in the editor

When you click in the Editor, you have to select first the file you want to edit, and then, this window is opened:



-Show All File Parameters: will show us all the parameter values, even the ones that we cannot modify. If it remains unselected, the software will show only the ones which can be modified. The values that cannot be modified are shown in grey.

-Help: document with the User´s Manual chapter of this Module will be displayed.

-With the **Select File** button, the user selects the FCS file that needs to be modified.

Once the editor has opened the file, it displays a table with a line for each text parameter in the TEXT section of the FCS file. For each of these parameters, the program shows the key at the left of the line, and the value at the right. The keys cannot be modified, and the text that can be modified is shown in white in the right column. To modify one of them, the user only has to double-click its cell and type the new text.

Save As…: Once the user has performed the appropriate modifications, it is possible to save the result to disk through this option. It is strongly recommended that the user saves it to a new file instead of rewriting the original one. Non-standard parameters are not guaranteed.

Main Menu: At any moment, the user can return to the Main Menu of the program by using this option.

9. FILE EXPORT

9.1. Introduction

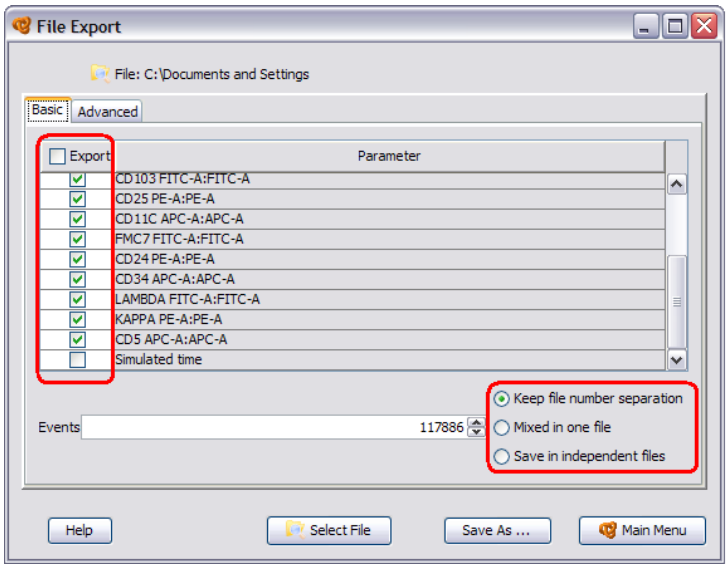
In this module, there are several options available to save to disk the results of the program, possibly with some additional information, in export formats readable by external analysis or database software, to facilitate data analysis.

There is also an access to *File Export* option from the *Manual Analysis* menu, which is more flexible to export only a group of visible gated events (see section 4.13.1.6.)

9.2. Available commands in the File Export window

When you click in the Export, you have to select first the file you want to export, and then, the Export window is opened.

BASIC FUNCTIONS



The user can export only the parameters which are necessary for the analysis, avoiding extra memory requirements resulting from unnecessary information. Initially, all the parameters are selected for export. If the user wants to eliminate some of these parameters, unclick the parameter to eliminate it from the export.

Simulated time: Last parameter to export is always simulated time. If you activate this checkbox, your export file will contain the chronology (simulated time) of the acquisition of the events/tubes as a new parameter, and it will be possible to represent this simulated time against any other parameter. Notice that in a merged file you already have the parameter "File Number" as the chronology of the tubes, then, is not necessary to add the simulated time parameter.

Events: This box allows the user to decrease the number of events selected to export.

If the user is trying to export a merged file, Infinicyt™ will give you the option to **keep the file number separation** (will maintain the separation in the original files), or **mix all the events in a file**, without separation between original files. Exported events will be a representative sample of the original data file.

A merged file can also be exported **in independent files** as they were originally.

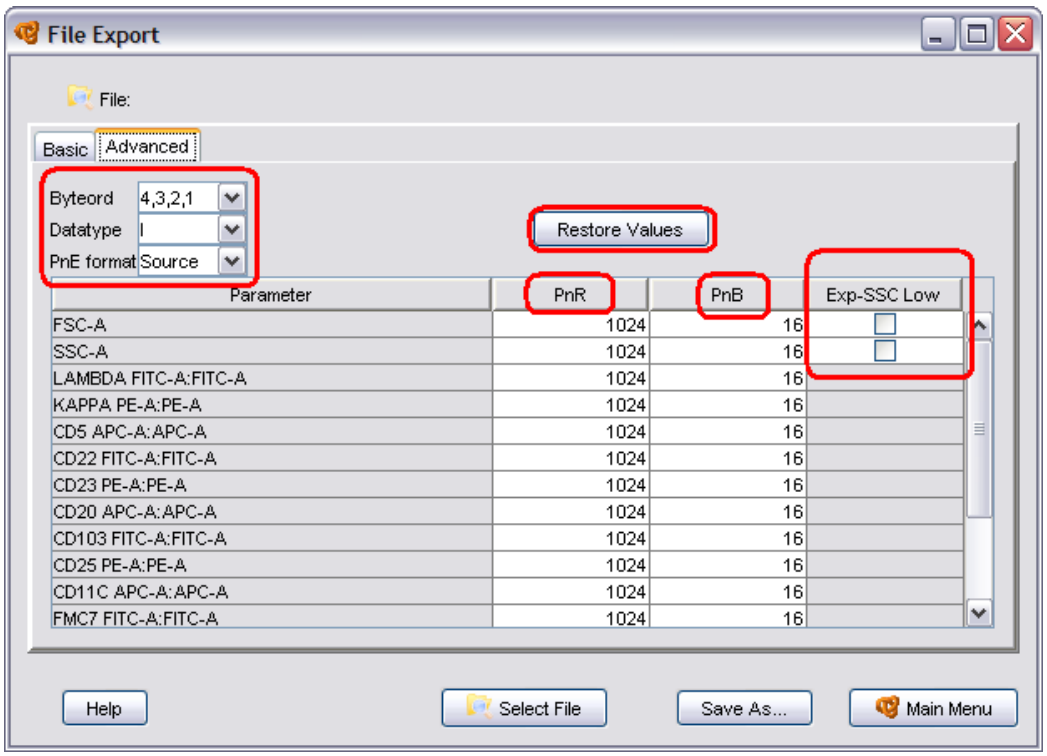
Help: Document with the User's Manual chapter of this Module will be displayed.

Select file: Allows changing the file you want to export, and the name of the file will appear on the top of the screen.

Save as: Once the user has selected the appropriate export options, if you click this button data currently opened will be saved to a disk file with the characteristics indicated by the selected options.

Main menu: At any moment, the user can return to the *Main Menu* of the program by using this option.

ADVANCED FUNCTIONS



Byteord, **Datatype** and **PnE Format:** These parameters are available for the user to obtain compatibility with other formats of *Flow Cytometry Standards.* In principle, there is no need to modify them, unless you want to change to a different format.

Restore Values: This button gives the opportunity of recovering the original data file values.

PnR: This value indicates the range of this parameter.

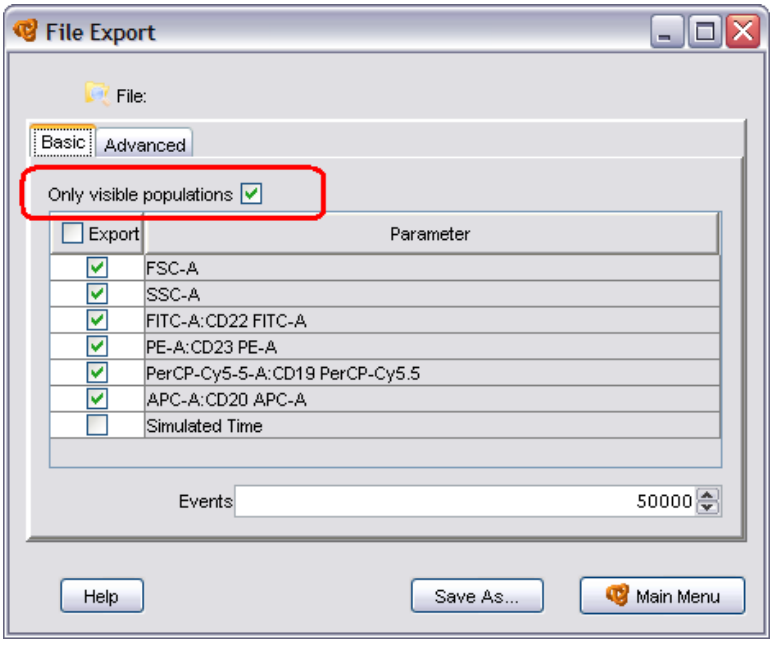
PnB: This value indicates the size of each value in the hard disk (in bits).

Exp-SSC Low: If you click this checkbox, a mathematical transformation will be applied to the parameter chosen of your export file to improve the separation between populations in the lower part of the scale.

9.3. Export a population from the Manual Analysis

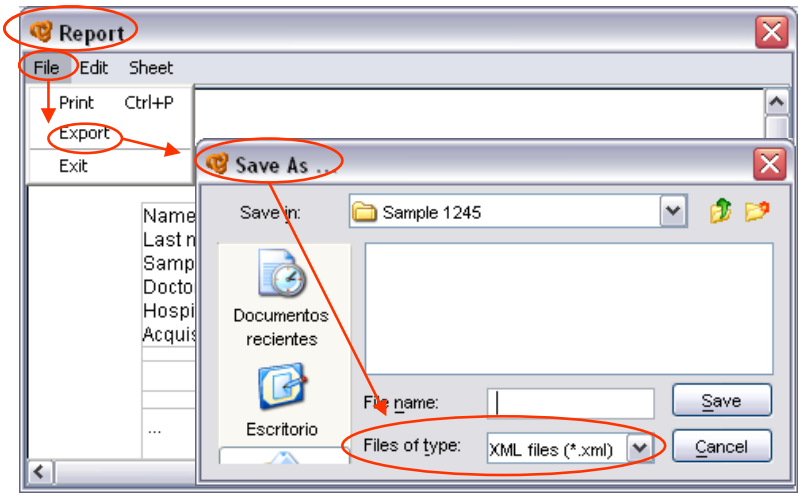
From the Main Screen the **File Export** option will consider the export of a whole file. If the user is interested on exporting only one or more populations, then, the Export option must be open from the *Analysis Window*, in the option **File / Export (Ctrl+E)**.

In case the user wants to export only the events included on a given population the export must be done from the Manual Analysis, and once the population is classified, and visible, go to the option **File/ Export.** In this dialogue box you will find the option **"export only visible populations"** which is not present in the export window obtained from the main menu. Through this option, only visible events in the file analyzed will be exported to the new data file.



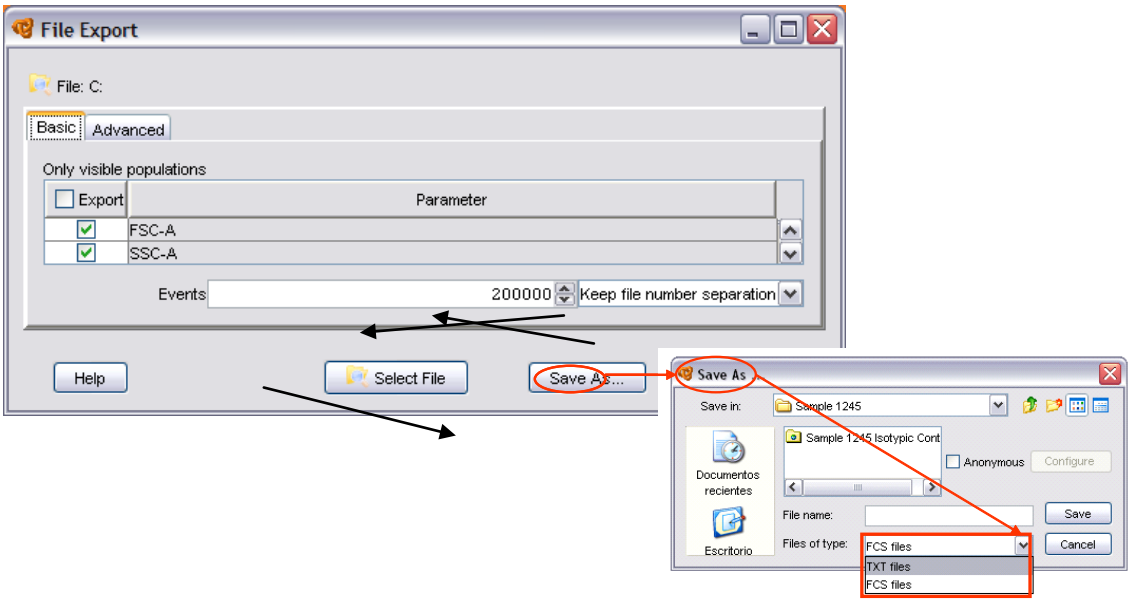
9.4. Export files in XML format

The Extensible Markup Language (XML) is a standard format of files compatible with all data base. Infinicyt™ gives the option to save the results of the report in this format and this can be done in both of the export option.



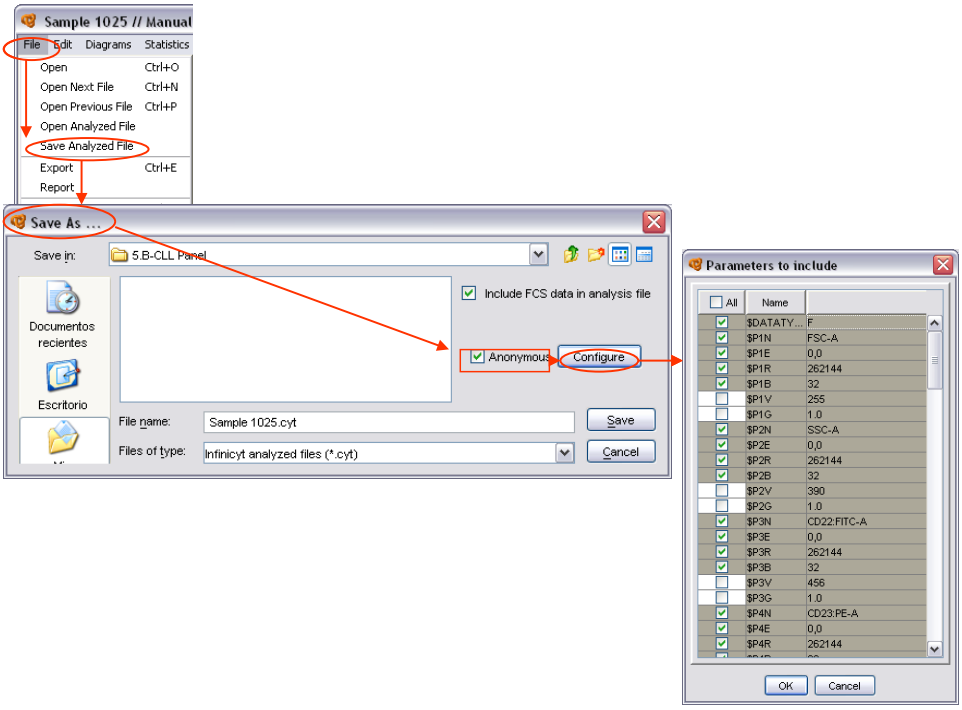
9.5. Export files in TXT format

In this version it is possible to export the results in TXT format as well, just choosing this option in “Files of Type” when you save as the results of the Export.



9.6. Export files in Anonymous format.

When you export both in FCS or TXT format, you will have the option to activate the checkbox “Anonymous”. You will have to click Configure to choose the information to delete (name of the patient, code number, date…). The parameters to include/exclude will be saved in the program configuration for next times, so you don’t have to configure it any time you repeat it. The anonymous option is also available when you save an analyzed file.



10. ADVANCED ANALYSIS

This option is not available in the Basic Version of the software.

In this Module, different Advanced Analysis will be included on each update. In this version, Automatic analysis for **BCR-ABL fusion protein kit** is available.

10.1. BCR-ABL Analysis Introduction

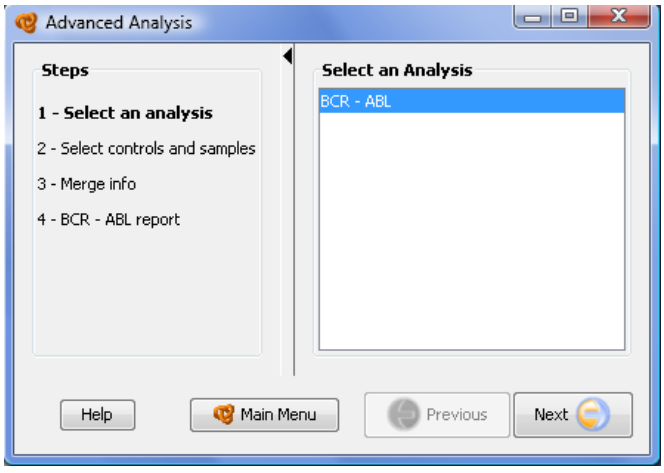
Philadelphia translocation is a specific chromosomal abnormality. It is the result of a reciprocal translocation between chromosome 9 and 22, and is specifically designated t (9;22)(q34;q11). The result, is a fusion gene created with a part of the *BCR* ("breakpoint cluster region") gene from chromosome 22 (region q11) to the *ABL* gene on chromosome 9 (region q34).

Within EUROFLOW PROJECT, it has been developed an automatic system to study the presence of BCR-ABL fusion proteins with BCR-ABL Protein Kit.

This Module is available on the Advanced Module, and it can be analyzed trough a wizard in the Advance Analysis from the Main Screen.

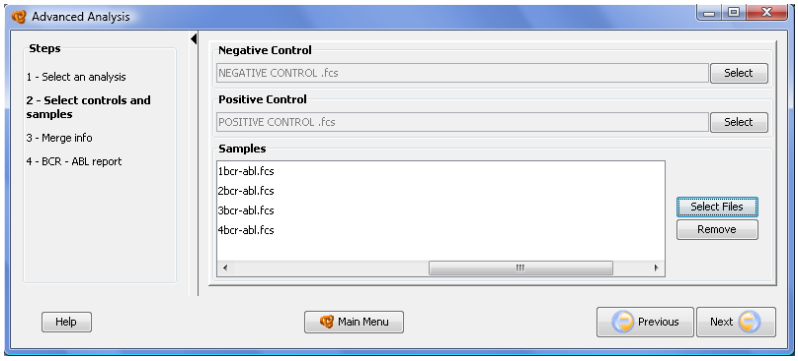
10.2. BCR-ABL Wizard

STEP 1: SELECT AN ANALYSIS



In this screen, the user will have to choose which of the Advance Analysis sections wants to follow. In this version, only BCR-ABL is available, and in future versions, more analysis will be included in this part. When the BCR-ABL is selected, click **Next.**

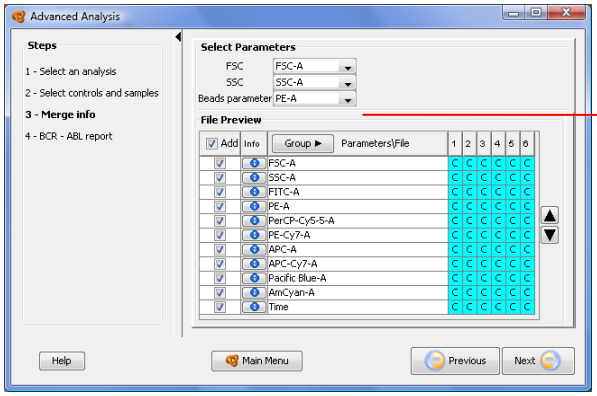
STEP 2: SELECT CONTROLS AND SAMPLES.



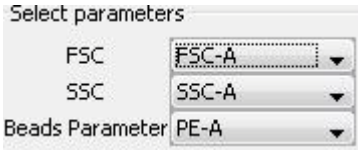
In this step, the user has to **Select** a negative control sample, **Select** a Positive control sample, and finally in the Samples box, the User can Select as many files as he/she wants to analyze. When negative, positive and at least one sample is uploaded, then it is possible to select the button **Next** to continue the analysis.

STEP 3: MERGE INFO

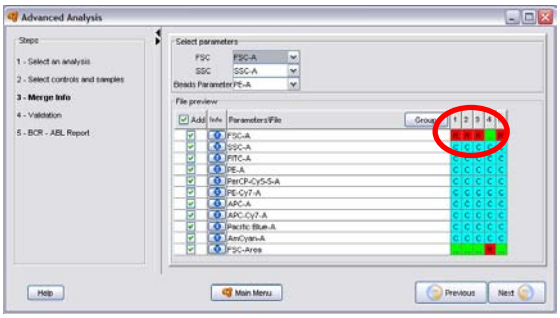
It is showed a matrix with info about the files and the parameters. In this step, it is possible to deselect parameters if you consider that are not necessary.



It is **very important** to select the correct **Beads Parameter**, this beads parameter comes from the fluorescence where the beads were measured. In this case is –PE.



The software validates if the parameters FSC, SSC and the Beads Parameter are common to all files. If there is something wrong it will show an error message.

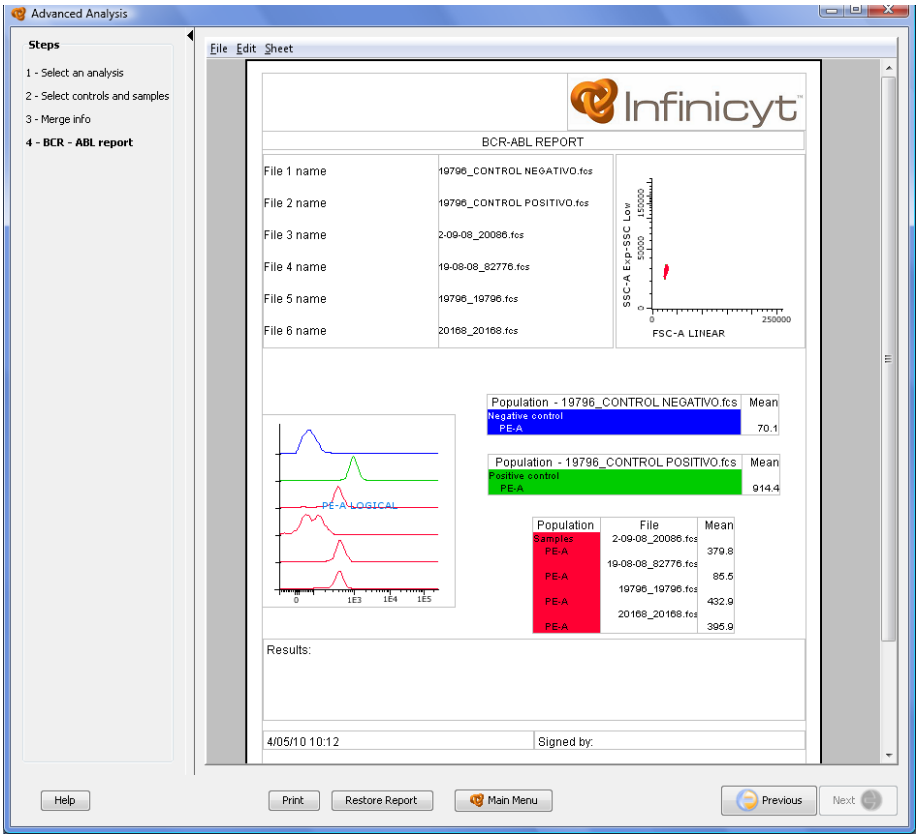


Then, in case there are discrepancies between common parameters we can use the dictionary to solve the discrepancies. See Dictionary tool explained on section 6.4.

Once this is done, you can click Next.

STEP 4: BCR-ABL REPORT

The software will show you the result of the analysis on a report. The software shows you a report by default, but it can be customized by the user.



CONTROL COMMANDS

Ctrl+ A = All Graphics to Front

Ctrl+ B = Close All Windows

Ctrl+ D = New Diagram

Ctrl+ E = Export

Ctrl+ G = Configure Profile

Ctrl+ I = File Properties

Ctrl+L = Duplicate Graph

Ctrl+ N = Open Next File

Ctrl+ O= Open

Ctrl+ P = Open Previous File

Ctrl+ R = Reset Analysis

Ctrl+ Y = Redo

Ctrl+ Z = Undo

Ctrl+ 0 = Delete all Selections

Ctrl+ T= Tile of diagrams

Ctrl + = Maximize Window

Ctrl - = Minimize Window

Ctrl+1,2,3… = Select File 1,2,3…